Lincoln University
Te Whare Wānaka o Aoraki
CHRISTCHURCH • NEW ZEALAND

Lincoln University Digital Thesis

Copyright Statement

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

This thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- you will use the copy only for the purposes of research or private study
- you will recognise the author's right to be identified as the author of the thesis and due acknowledgement will be made to the author where appropriate
- you will obtain the author's permission before publishing any material from the thesis.

Neuroendocrine Regulation of Dry Matter Intake in Grazing Dairy Cows.

A thesis

submitted in partial fulfilment

of the requirements for the Degree of

Doctor of Philosophy

at

Lincoln University

by

Angela Joy Sheahan

Lincoln University 2014

Abstract

Abstract of a thesis submitted in partial fulfilment of the

requirements for the

Degree of Doctor of Philosophy.

Neuroendocrine Regulation of Dry Matter Intake

in Grazing Dairy Cows.

by

Angela Joy Sheahan

Supplementary feeds are offered to grazing dairy cows to increase dry matter and metabolizable energy intakes; however, offering feed supplements reduces pasture dry matter intake, a phenomenon known as substitution. The objective of this research was to understand variations in grazing behaviour in pasture-fed cows and the effects of supplementation on grazing time and feeding intake rate throughout the day and to investigate humoral profiles of factors known to be associated with intake regulation in monogastric species and quantify their role in ruminant species. Grazing occurred predominately during daylight hours, with minimal grazing during the hours of darkness. Distinct grazing bouts were evident post sunrise and pre-sunset. Supplementation reduced time spent grazing; however, this was an accumulation of reduced grazing time throughout the day and was not restricted to the period following the consumption of supplement, as fundamentally, the profile of grazing behaviour in supplemented cows followed the same pattern as unsupplemented cows. The effects of

supplementation on time spent grazing differed depending on the time of day. Time spent grazing linearly reduced with increasing supplement in the a.m., whereas, time spent grazing was unaffected by supplementation during the pre-sunset grazing bout, irrespective of supplement level or timing of sunset. The differences in grazing behaviour during the major post-sunrise and pre-sunset grazing events lead to the hypothesis that different factors regulate dry matter intake at these times. In the a.m., products of digestion and associated physiological factors regulate grazing behaviour. Whereas, in the p.m., environmental cues (i.e. sunset) override physiological signals that regulate grazing behaviour in the a.m. to ensure maximal grazing occurs prior to darkness, irrespective of supplementation or energy balance status.

Humoral profiles of factors implicated in intake regulation in monogastric species were similar in the dairy cow. Humoral factors associated with a fasted or pre-prandial state were elevated and declined after meal initiation, whereas, factors indicating a change from a negative to a positive energy state increased after meal initiation. Despite the similar humoral profiles, the profile of plasma ghrelin during the major p.m. feeding event differed from its reported decrease in concentration after feeding, establishing a unique profile for ghrelin. Plasma ghrelin increased in the p.m. despite intensive grazing/feeding and cows being in a positive energy state prior to the p.m. feeding event, which had not been previously reported in ruminant species. The increase in ghrelin was coincident with an increase in the intensity of grazing/feeding that lead to the hypothesis that ghrelin increases in diurnal species ensuring animals maximise dry matter intake prior to darkness, which is a major environmental cue to cease grazing/feeding.

Key words: dairy cow, grazing behaviour, timing of supplementation, supplementary feeding, substitution rate, humoral, ghrelin,

Acknowledgements

Firstly, I wish to express my extreme appreciation to my DairyNZ supervisor, Dr John Roche, for his continued support and guidance, not only throughout my PhD research, but also during previous years as a research technician.

I wish to recognise the financial, academic and technical support offered to me by DairyNZ. In particular, the farm staff, technical team, academic committee, Barbara Dow, and Dr Jane Kay. I would also like to thank Dr Ray Boston for his patience and friendship.

I wish to express my sincere appreciation to all those not mentioned, who have contributed to this thesis and supported me in one way or another.

Finally, I would like to acknowledge the most important people in my life – my family. All of whom have been a constant source of strength and encouragement, through both the good and tough times.

Table of Contents

Abstract i

Acknowledgements iii

Table of Contents iv

List of Tables x

List of Figures xii

List of Abbreviations xv

Published Sections of this Thesis. xix

Chapter 1 Introduction 1

1.1 Ruminant Agriculture in New Zealand 1

1.2 Importance of Dry Matter Intake 4

Chapter 2 Literature Review 6

2.1 The Ruminant 6

2.1.1 Ruminant Evolution 6

2.1.2 Anatomy of the Ruminant Digestive Tract 8

2.1.2.1 Reticulo-rumen 10

2.1.2.2 Omasum 11

2.1.2.3 Abomasum 12

2.1.2.4 Small and Large Intestine 12

2.1.3 Unique Aspects of Digestion in the Ruminant 14

2.1.3.1 Reticulo-rumen Motility 14

2.1.3.1.1 Primary Contractions 14

2.1.3.1.2 Secondary Contractions 15

2.1.3.2 Rumination 15

2.1.3.3 Microorganisms 16

2.1.3.3.1 Fungi 17

2.1.3.3.2 Protozoa 17

2.1.3.3.3 Bacteria 17
2.1.4 Rumen Fermentation 19
2.1.4.1 Carbohydrates 19
2.1.4.1.1 Acetate 21
2.1.4.1.2 Propionate 21
2.1.4.1.3 Butyrate 22
2.1.4.1.4 Other small VFA 23
2.1.5 Protein 23
2.1.6 Lipids 26
2.1.6.1 Ruminal Absorption 28
2.1.7 Gluconeogenesis 28
2.2 Grazing Behaviour 29
2.2.1 Environment 31
2.3 Intake Regulation in the Ruminant 32
2.3.1 Physical Factors 33
2.3.1.1 Presentation of Feed 33
2.3.1.2 Supplementation 35
2.3.1.3 Cow Genetic Merit 36
2.3.1.4 Physiological state 36
2.3.2 Physiological Factors 38
2.3.2.1 Hunger and Satiety 39
2.3.3 Anatomy of the Brain Pertaining to Intake Regulation 39
2.3.3.1 Blood Brain Barrier 39
2.3.3.2 Median Eminence 40
2.3.3.3 The Hypothalamus 40
2.3.3.4 Brainstem - Vagus Nerve 43
2.3.4 The Central Regulation of Food Intake 44
2.3.4.1 Neurotransmitters 45
2.3.4.2 Metabolic Enzymes 45
2.3.5 Integration of peripheral signals 46
2.3.5.1 In a Fasted State 51

2.3.5.2 In a Fed State ... 54
2.3.6 Intake Regulation in Ruminant Species ... 57

Chapter 3 Genetic Strain and Diet Effects on Grazing Behaviour, Pasture Intake and Milk Production. ... 60

3.1 Abstract ... 60
3.2 Introduction ... 61
3.3 Materials and Methods ... 63
3.3.1 Experimental Design ... 63
3.3.2 Genetic strains ... 63
3.3.3 Pasture Management and Supplementary Feeding Treatments ... 64
3.3.4 Milk Production ... 64
3.3.5 Grazing Behaviour ... 65
3.3.6 Pasture Intake Measurements ... 65
3.3.7 Statistical Analysis ... 66
3.4 Results ... 66
3.5 Discussion ... 73
3.6 Conclusions ... 76

Chapter 4 Timing of Supplementation Alters Grazing Behaviour and Milk Production Response in Dairy Cows ... 77

4.1 Abstract ... 77
4.2 Introduction ... 78
4.3 Materials and Methods ... 78
4.3.1 Experimental design ... 79
4.3.2 Pasture and Concentrate Supplement ... 79
4.3.3 Pasture Dry Matter Intake ... 81
4.3.4 Milk Production ... 81
4.3.5 Grazing Behaviour ... 81
4.3.6 Statistical Analysis ... 82
4.4 Results ... 82
4.5 Discussion ... 86

4.6 Conclusion 89

Chapter 5 Diurnal Patterns of Grazing Behaviour and Humoral Factors in Supplemented Dairy Cows 90

5.1 Abstract 90
5.2 Introduction 91
5.3 Materials and Methods 92
5.3.1 Experimental design 93
5.3.2 Pasture management and supplementary feeds 93
5.3.3 Animal Measurements 93
5.3.3.1 Grazing Behaviour 93
5.3.3.2 Jugular Catheters and Blood Sampling 94
5.3.3.3 Blood 94
5.3.4 Statistical Analysis 95
5.4 Results 95
5.5 Discussion 112
5.6 Conclusion 114

Chapter 6 Carbohydrate Supplements and their Effects on Pasture Dry Matter Intake, Feeding Behaviour and Humoral Factors. 115

6.1 Abstract 115
6.2 Introduction 116
6.3 Materials and Methods 117
6.3.1 Experimental Design 117
6.3.1.1 Feed Management 118
6.3.2 Pasture and Animal Measurements 118
6.3.2.1 Pasture and Supplement Intakes and Feeding Behaviour 118
6.3.2.2 Jugular Catheter and Blood Sampling 120
6.3.2.3 Plasma Hormone and Metabolite Assays 121
6.3.3 Statistical Analysis 122
6.4 Results 122
6.4.1 Dry Matter Intake and Feeding Behaviour 122

6.4.2 Profile of Change in Humoral Factors Associated with DMI.............. 125
6.4.3 Supplementation Affects Humoral Intake Regulatory Factors Profiles 126
6.5 Discussion 136
6.5.1 Feeding behaviour and Pasture DMI - a.m. vs p.m. 136
6.5.2 Humoral Factors - a.m. vs p.m.. 137
6.5.3 Effect of Supplement on Feeding Behaviour and Pasture DMI 139
6.5.4 Effect of Supplement Type on Humoral Factors 141
6.5.5 Substitution and Humoral Factors 142
6.6 Conclusion... 143

Chapter 7 General Discussion and Conclusions. 144

7.1 Grazing Behaviour 144
7.2 Humoral Factors... 146
7.3 Conclusions of this Thesis... 150

Appendix A Orexigenic and Anorexigenic Signals Regulating Intake. 151

A.1 Central Intake Regulation Factors... 152
A.1.1 Orexigenic Peptides 152
A.1.1.1 Neuropeptide Y... 152
A.1.1.2 Agouti-related Protein.. 153
A.1.1.3 Orexins... 154
A.1.2 Anorexigenic Peptides 154
A.1.2.1 Melanocortin... 154
A.1.2.2 Cocaine-and amphetamine-regulated transcript 155
A.2 Peripheral Intake Regulatory Signals.. 156
A.2.1 Gastro Intestinal... 156
A.2.1.1 Orexigenic... 156
A.2.1.1.1 Ghrelin 156
A.2.1.1.2 Glucagon-like peptide 1.. 159
A.2.1.1.3 Gastric Inhibitory Peptide... 160
A.2.1.1.4 Peptide YY 161
A.2.1.1.5 Cholecystokinin 161

A.2.2 Pancreas 162
A.2.2.1 Insulin 163
A.2.2.2 Glucagon 165
A.2.2.3 Pancreatic Polypeptide 166
A.2.3 Adipose Tissue 167
A.2.3.1 Leptin 167
A.2.3.2 Adiponectin 169
A.2.3.3 Resistin 170

References 171

List of Tables

Table 2.1 Daily time spent grazing and ruminating (min) for North American (NA) and New Zealand (NZ) pasture-fed cows. 31

Table 2.2 The defined regions, area and respective nuclei within the hypothalamus 41

Table 2.3 Summary of key gastrointestinal neuropeptides...48

Table 2.4 Summary of key neuropeptides involved in intake and there effects on feed intake in a normal, intracerebroventricular administered and gene knockout scenarios. 49

Table 3.1 Pasture and total DMI (kg DM/day), milk and component yield (kg/day) and milk composition (%) during peak, mid, and late lactation for New Zealand (NZ) and North American (NA) cows offered 0, 3, or 6 kg DM/day concentrates. 69

Table 3.2 Summary of grazing and ruminating behaviour during peak, mid, and late lactation for New Zealand (NZ) and North American (NA) cows offered 0, 3, or 6 kg DM/day concentrates. Daily grazing and ruminating time, time spent grazing during four distinct periods of the 24 h period, and length of first grazing bout following a.m. and p.m. concentrate allocation. 70

Table 4.1 Chemical composition of bulked a.m. and p.m. pasture samples and concentrate supplement offered. 80

Table 4.2 Summary of milk production, milk components and estimated pasture intake for cows on pasture only (PASTURE), pasture + a.m. supplement only (AMSUP), and pasture + p.m. supplement only (PMSUP). 83

Table 4.3 Summary of grazing, ruminating and idling behaviour. Daily total grazing, ruminating and idling times, time spent grazing, ruminating and idling during four time blocks (TB) during a 24 h period, and length of major grazing bout following a.m. and p.m. milking for cows on pasture only (PASTURE), pasture + a.m. supplement only (AMSUP), and pasture + p.m. supplement only (PMSUP). 85

Table 6.1 Chemical composition of pasture samples fed during the a.m. and p.m. measurement periods and the starch- and fibre based concentrate supplement fed in equal portions at a.m. and p.m. milking. 120

Table 6.2 Pasture and total DMI (kg DM) and the proportion of time spent in eating, idle, and ruminating for 240 min after a.m. and p.m. milking. Cows (5/treatment) received pasture only (PASTURE), pasture plus 3.5 kg/day DM starch-based concentrate (STARCH), or pasture plus 4.4 kg/day DM fibre–based concentrate (FIBRE). 124

List of Figures

Figure 1.1 New Zealand regional distribution of dairy cows in 2010-2011....................3
Figure 2.1 Diagram of ruminant digestive system illustrating the movement of feed. Green indicates feed eaten, red indicates regurgitated bolus (ruminating) back into the mouth, blue indicates the re-masticated bolus back into the digestive system and black indicates passage into small intestine. 9
Figure 2.2 Diagram of internal view of the rumen indicating the subdivision into the (a) dorsal, (b) ventral, (c) caudodorsal, and (d) caudoventral. 11
Figure 2.3 Overview of carbohydrate metabolism in the dairy cow. 20
Figure 2.4 Proportions of VFA produced in the rumen when pH falls from 7.0. 22
Figure 2.5 Overview of protein metabolism in the dairy cow. 25
Figure 2.6 Overview of fat metabolism in the dairy cow. 27
Figure 2.7 Dry matter intake, milk yield and live weight in a Friesian cow during the lactation cycle...38
Figure 2.8 Location of hypothalamus within the brain (a), anatomical structure of nuclei within the hypothalamus (b)...42
Figure 2.9 Diagram illustrating hypothalamic and peripheral activity in a fasted or pre-prandial state. In a fasted or pre-prandial state, the stomach releases increased concentrations (conc) of ghrelin that enter circulation and cross the blood brain barrier via the median eminence and binds to its receptor on the NPY/AgRP neurone, within the arcuate nucleus (Arc) in the hypothalamus. This stimulates the expression and release of NPY and AgRP. Increased NPY concentration stimulates second order neurones in the LHA and PVN to release the orexigenic neuropeptides orexin and MCH, whilst inhibiting release of the anorexigenic neuropeptides CRH and TRH from the PVN, respectively. Additionally, the low circulating concentration of insulin and leptin also stimulate the release of AgRP that binds to the MC receptor on the POMC neurone, inhibiting the release of α-MSH. The simultaneous

stimulatory and inhibitory activity from peripheral sites to the NTS, via the vagus nerve, project to the LHA stimulating release of MCH and orexin, and to the VSC in the thalamus that brings about the perception of hunger. These combined hypothalamic and peripheral signals induce feed intake. 52

Figure 2.10 Diagram illustrating hypothalamic and peripheral activity in a fed state. After the consumption of food, the concentrations of leptin and insulin increase, while ghrelin concentration decreases. The increased concentrations of leptin and insulin stimulate POMC neurones, and inhibit the NPY/AgRP neurone promoting release of the anorexigenic neuropeptide α-MSH (derived from POMC) that binds to its receptor in the PVN stimulating release of anorexigenic neuropeptides CRH and TRH, inhibiting neuropeptide release from the LHA. The simultaneous stimulatory and inhibitory activity from peripheral sites to the NTS, via the vagus nerve, project to the LHA inhibit release of MCH and orexin, and to the VSC in the thalamus that brings about the sensation of fullness and satiety. These combined hypothalamic and peripheral signals terminate feed intake. 55

Figure 3.1 Diurnal profile of cows grazing during peak, mid, and late lactation when offered 0, 3 or 6 kg DM/day concentrate (0 kg DM/day = dotted line, 3 kg DM/day = solid line and 6 kg DM/day = dashed line). Shading represents milking time, and vertical dashed lines represent sunrise and sunset. 71

Figure 3.2 Diurnal profile of rumination in cows during peak, mid, and late lactation when offered 0, 3 or 6 kg DM/day concentrate (0 kg DM/day = dotted line, 3 kg DM/day = solid line and 6 kg DM/day = dashed line). Shading represents milking time, and vertical dashed lines represent sunrise and sunset. 72

Figure 5.1 Diurnal profiles of percentage of cows (a) grazing, (b) ruminating, and (c) idling behaviour for 17 dairy cows offered 4.4 kg/DM per day of a

concentrate supplementary feed in equal portions at a.m. and p.m. milking. Vertical solid lines represent sunrise (0620 h) and sunset (1810 h). Dashed lines represent a.m. and p.m. milking. 98

Figure 5.2. Diurnal profiles of grazing behaviour for 17 cows (proportion of cows grazing; shaded) and averaged circulating humoral factors considered to have a regulatory role in intake regulation; (a) Ghrelin, (b) Insulin, (c) Glucose, (d) NEFA, (e) Growth Hormone, (f) Leptin, (g) GLP-1, (h) Glucagon, (i) IGF-1 for 10 of the 17 cows offered 4.4 kg/day of a concentrate supplementary feed in equal portions at a.m. and p.m. milking. Vertical dashed lines represent sunrise (0620 h) and sunset (1810 h). Standard error of the mean bars is included. 102

Figure 6.1 Average plasma concentrations (log10 transformed if required) wit fitted spline for 15 cows (5/treatment) offered pasture only (PASTURE ___x___), pasture plus 3.5 kg/day DM starch-based concentrate (STARCH ….◊….), or pasture plus 4.4 kg/day DM fibre–based concentrate (FIBRE ---□---) during a 240 min measurement period after a.m. and p.m. milking; (a) ghrelin a.m. (b) ghrelin p.m. (c) insulin a.m. (d) insulin p.m. (e) NEFA a.m. (f) NEFA p.m. (g) glucose a.m. (h) glucose p.m. Error bars are the standard error of the mean. Time and Treatment (Trt) effects are presented. 127

Figure 6.2 Average plasma concentrations (log_{10} transformed if required) with fitted spline for 15 cows (5/treatment) offered pasture only (PASTURE —x—), pasture plus 3.5 kg/day DM starch-based concentrate (STARCH ····◊····), or pasture plus 4.4 kg/day DM fibre–based concentrate (FIBRE ---□---)during the 240 min measurement period after a.m. and p.m. milking; (a) BHBA a.m. (b) BHBA p.m. (c) NPY a.m. (d) NPY p.m. (e) Glucagon a.m. (f) Glucagon p.m. Error bars are the standard error of the mean. Time and Treatment (Trt) effects are presented. 132

List of Abbreviations

ACC	Acetyl CoA carboxylase
ADF	Acid detergent fibre
AgRP	Agouti-related protein
AMPK	Adenosine 5'-monophosphate-activated protein kinase
ANOVA	Analysis of variance
Arc	Arcuate nucleus
ATP	Adenosine triphosphate
β	Beta
BBB	Blood brain barrier
BCS	Body condition score
BHBA	β-hydroxybutyrate
BW	Body weight
CART	Cocaine and amphetamine regulated transcript
CCK	Cholecystokinin
CNS	Central nervous system
CNCPS	Cornell net carbohydrate and protein system
CoA	Co enzyme A
CO_2	Carbon dioxide
CPIR	Cephalic phase insulin response
CP	Crude protein
CRH	Corticotropin releasing hormone
CV	Coefficient variation
d	Day
DMI	Dry matter intake
DM	Dry matter
DMH	Dorsomedial nucleus
DMV	Dorsomotor nucleus of the vagus

DPP-IV	Dipeptidyl peptidase four
EB	Energy balance
EBV	Estimated breeding values
FA	Fatty acid
GABA	ϒ-amino butyric acid
GH	Growth hormone
GHS-R	G-protein coupled receptor
GI	Gastrointestinal
GLP-1	Glucagon-like peptide-1
GMT	Greenwich mean time
h	Hour
HF	Holstein Friesian
HOT	Hepatic oxidation theory
ICV	Intracerebroventricular
IGF-1	Insulin-like growth factor 1
IV	Intravenous
Kg	Kilogram
LAB	Liquid associated bacteria
LCFA	Long chain fatty acid
LHA	Lateral hypothalamic area
LP	Lipoproteins
MC3-R	Melanocortin 3 receptor
MC4-R	Melanocortin 4 receptor
MCH	Melanin concentrating hormone
MC	Melanocortin
MeE	Median eminence
ME	Metabolisable energy
MP	Metabolisable protein
MR	Milk response
min	Minute
MJ	Mega joules

mL	Millilitre
mm	Millimetre
MP	Metabolisable protein
mRNA	Messenger ribonucleic acid
α-MSH	α- melanocyte-stimulating hormone
NA	North American
NDF	Neutral detergent fibre
NEFA	Non-esterified fatty acid
NFF	Non-forage fibre
NPN	Non protein nitrogen
NPY	Neuropeptide Y
NSC	Non-structural carbohydrates
NTS	Nucleus solitary tract
NZ	New Zealand
OM	Organic matter
OXM	Oxyntomodulin
PA	Pasture allowance
POMC	Proopiomelanocortin
PVN	Paraventricular nucleus
PYY	Peptide tyrosine tyrosine
REML	Residual maximum likelihood
RDP	Rumen degradable protein
SAB	Solid associate bacteria
SCN	Suprachiasmatic nucleus
SED	Standard error of the deviation
SH	Sward height
SR	Substitution rate
TB	Time block
TCA	Tricarboxylic acid cycle
TG	Triglyceride rich
TMR	Total mixed ration

TRH	Thyrotropin-releasing hormone
UDP	Undegradable protein
VFA	Volatile fatty acid
VFI	Voluntary food intake
VMH	Ventromedial nucleus
VSC	Visceral sensory complex
WSC	Water soluble carbohydrates
Yr	Year

Published Sections of this Thesis.

Chapter 3

Sheahan, A. J., E. S. Kolver and J. R. Roche. 2011. Genetic strain and diet effects on grazing behaviour, pasture intake, and milk production. Journal of Dairy Science. 94: 3583-3591

Chapter 4

Sheahan, A. J., S. J. Gibbs and J. R. Roche. 2013. Timing of supplementation alters grazing behaviour and milk production response in dairy cows. Journal of Dairy Science . 96: 477-483.

Chapter 5

Sheahan, A. J., R. C. Boston and J. R. Roche. 2013. Diurnal patterns of grazing behaviour and humoral factors in supplemented dairy cows. Journal of Dairy Science. 96: 3201-3210

Chapter 6

Sheahan, A. J., J. K. Kay and J. R. Roche. 2013. Carbohydrate supplements and their effects on pasture dry matter intake, feeding behavior, and blood factors associated with intake regulation. Journal of Dairy Science. 96: 7818–7829

Chapter 1
Introduction

Cattle, sheep and goats comprise about 95% of domesticated ruminant animals in modern times (Clutton-Brock, 1999). The goat was the first livestock species to be domesticated (ruminant or non-ruminant); this occurred approximately 10,000 B.C. in the highlands of western Iran (Zeder and Hesse, 2000). The goat was domesticated to supply meat to human populations, where hunting had depleted large prey populations (Clutton-Brock, 1999). Most of the other domesticated ruminants (sheep, water buffalo, yak, European and Zebu cattle) became domesticated by 2,500 B.C. in either the near East or southern Asia. The reason for the domestication of various species varied greatly, including meat, milk, transportation, barter and sacrifice (Clutton-Brock, 1999).

The biological purpose of lactation in the cow is to provide nourishment to her newborn calf until their rumen has developed to a stage when the calf can meet its own nutritional requirements. However, for thousands of years humans have exploited this to provide quality food. Dairy industries have developed the cow's abilities in three areas:

1. produce more milk than would be needed to sustain a calf,
2. become a non-seasonal breeder,
3. secrete milk without the presence of the calf (Holmes et al., 2003).

1.1 Ruminant Agriculture in New Zealand

Statistics in this section (1.1) were sourced from: Statistics New Zealand Agricultural Census 2007, ANZIC 2006, New Zealand Dairy Statistics 2010-2011.

For the last 100 years, agriculture has been New Zealand's largest sector of the economy and farming is the main export earner. The total land area of New Zealand is 26.8 million hectares and, of those, 14.7 million hectares is allocated to farming. Dairy

farming is part of a long and proud agricultural tradition in New Zealand. Dairy cattle were first imported by European settlers in the early 1800's to provide milk, butter and cheese for local supply. As early as 1846, only six years after the signing of the Treaty of Waitangi, the first exports began. In 1882, New Zealand exported the first refrigerated shipment of meat and butter to London.

Average herd size has increased from 124 cows in 1979-80 to 386 cows in 2010-11. In 2010-11, 17,339 million litres of milk was processed by dairy factories, with an average of 190 kg milk fat and 140 kilograms (**kg**) milk protein, or 3,829 litres of milk per cow. The majority of dairy herds (64%) are located in the North Island, with the greatest concentration (36%) in the Waikato region. Although, South Island dairy herds account for less than one quarter of the national total, they contain one third of all cows (Figure 1.1).

As of 30 June 2010, New Zealand was home to 32.5 million sheep and was the third largest producer of wool on a 'clean' basis in the world, accounting for 11% of world production. Along with wool exports, approximately 600,000 tonnes/year of sheep meat was exported, accounting for 55% of world trade and 75% of world lamb exports.

Beef cattle numbers were approximately 3.9 million. Few farmers in New Zealand dedicated themselves exclusively to beef production. In general, the raising and finishing of beef cattle is undertaken in conjunction with sheep farming. In addition, beef is produced from cull dairy cows and bobby calves. New Zealand exports 634,000 tonnes/year of beef and veal. Deer farming is increasing in New Zealand, with the country having become the worlds largest exported of farmed venison.

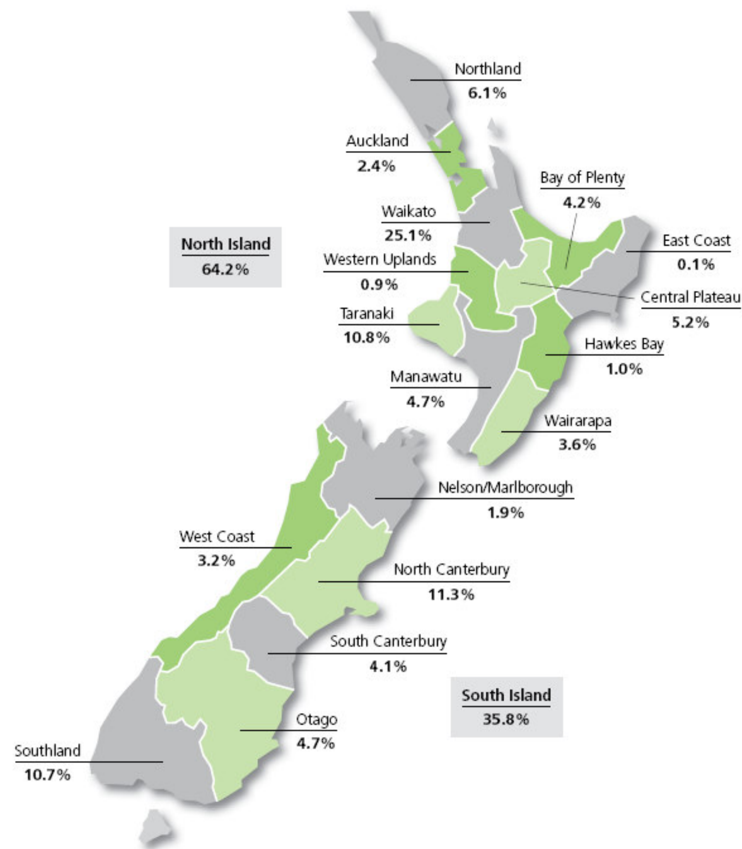


Figure 1.1 New Zealand regional distribution of dairy cows in 2010-2011.

Sourced from New Zealand Dairy Statistics 2010-11

New Zealand leads the world in dairy exports, accounting for over one third of international dairy trade. Ninety one percent of products from New Zealand farms are exported. New Zealand dairy products feed more than 100 million people worldwide, and the dairy industry directly contributes 25% of New Zealand's merchandise export earnings. Since its beginnings, dairy farming has expanded in New Zealand, producing 1.7 million tonnes/year of dairy products, to become the world's eighth largest milk producer.

Modern dairy industries worldwide have the common objective of producing high quality dairy products. However, despite this common objective, there are differences in on-farm milk production systems, due to the relative availability and costs of a variety of feeds, and the milk price paid to the farmers (Holmes et al., 2003). Few countries utilise pasture as a primary feed for dairy cows, with the majority of cows fed total mixed rations (**TMR**; Diouf, 1995). New Zealand's climate allows for the growth of high quality temperate pastures over much of the year; perennial pastures form the main source of nutrients and metabolisable energy for grazing animals (Penno, 2002). As pasture is routinely grazed, it provides an inexpensive but high quality feed (Horan et al., 2005); however, pasture-based dairy systems come with their challenges. For example, the amount and quality of pasture declines as it is consumed (Holmes et al., 2003). In comparison, the quality and quantity of the diet does not change in a TMR-based system. There are seasonal effects, such as droughts and floods, which can affect pasture growth, compared with a TMR, which is normally fed in housed facilities. Farmers plan calving such that peak herd demand coincides with the increased spring pasture growth to achieve maximum utilisation of pasture (Horan et al., 2004), with excess pasture harvested for future feed when pasture growth is less (Holmes et al., 2003). Specialist crops, such as maize, cereals, brassica species and lucerne are used in addition to pasture silage or hay crops, which can be fed when pasture supply is scarce.

1.2 Importance of Dry Matter Intake

Dry matter intake (**DMI**) is arguably the most important factor in animal production, indicative of the amount of nutrients available for production and, thereby, affecting gross feed conversion efficiency (i.e. nutrients directed to production-related processes relative to those directed to maintenance; Roche et al., 2007b). Many factors affect DMI, with many individual hypotheses reported to regulate DMI, based on physical fill of the reticulo-rumen (Mertens, 1994; Allen, 1996) or metabolic-feedback factors (Mertens, 1994; Illius and Jessop, 1996; Allen et al., 2009). Each hypothesis has

merit but it is most likely that the regulation of DMI is multifactorial in nature (Forbes, 2007).

Relatively low dry matter (**DM**) and metabolisable energy (**ME**) intakes are primary limitations to productivity in pasture-based dairy systems, resulting in nutrient intakes that are insufficient to match the milk production potential of the grazing dairy cow (Kolver and Muller, 1998). Supplementary feeds are often offered to grazing cows in an attempt to overcome these limitations (Stockdale, 1999; Bargo et al., 2003). However, total DMI does not increase by the amount of supplement consumed, as cows reduce their pasture DMI when offered supplementary feeds (Bargo et al., 2003); this phenomenon is known as substitution. The animal-related factors contributing to substitution are unclear, but they are very likely the same factors regulating the beginning and cessation of a meal.

Chapter 2
Literature Review

2.1 The Ruminant

2.1.1 Ruminant Evolution

The evolutionary success of ruminants relies on the ability to extract nutrition from low quality feeds (Webb, 1998). For the most part, the digestive system of ruminant animals is very similar to that of other mammals; however, the stomach differs considerably. In monogastric species, the stomach's functions are limited to temporary storage and preliminary mastication of the food into a liquid mass; little absorption of nutrients takes place, as most absorption is in the intestines (Frandson et al., 2006). The ruminant stomach is an evolutionary adaptation of a single stomach modified by expansion of the oesophageal region into three distinct areas, the rumen, reticulum and omasum, collectively known as the forestomach (Frandson et al., 2006). Within the forestomach, a symbiotic relationship exists between the ruminant animal and the microbes inhabiting it (Dobson et al., 1984). The ruminant provides an ideal environment for the microbes and, in return, microbes digest cellulose and other dietary components, providing an otherwise unavailable energy source for the ruminant (Church, 1993).

The first ruminant species evolved approximately 50 million years ago and were small, reclusive, forest-dwelling omnivores (Métais and Vislobokova, 2007). Their skull and dental morphology (low-crowned teeth, small incisors and long narrow skulls) were ideal for eating fruits, shoots and insects (Webb, 1998). Foregut fermentation and rumination was not extensive when the first ruminants emerged, but developed approximately 40 million years ago, as indicated by dental morphology (Janis, 1976) and molecular techniques (Jermann et al., 1995). Fossil records also indicate the ruminant has progressively increased in size over time (Janis, 1976).

Ruminants evolved in two ways:

1. foregut fermentation,
2. the ingestion of microbes as they pass through the gut (Kornegay et al., 1994).

Two hypotheses exist as to why this type of digestive adaptation developed. Previously, scientists thought that the ruminant could better escape predators because it devoured its food quickly and masticated later (Church, 1993). While this may have played a role, more importantly is that pre-digestion detoxifies secondary plant substances, which had begun to appear with the radiation of the angiosperms during the Miocene period (between 5-23 million years ago; Van Soest, 1994). The advantages of foregut fermentation are best realised when the animal is consuming meals of high fibre content, and it is likely that the foregut fermenters evolved in regions of poor pasture quality (Van Soest, 1994). The rapid spread of ruminants in the Miocene and Pliocene is concurrent with the spread of grasslands throughout the world (Hume, 1999).

The advantages of the ruminant digestive system are numerous:

- The highly lignified cell walls of poor quality forages pose less of a problem to foregut fermenters; because of regurgitation and repeated mastication, the contents of the cells are more easily accessed and utilised (Russell and Wilson, 1996).
- Some plant toxins are also degraded in the foregut via microbial fermentation, protecting the animal from harm (Hume, 1999).
- Ruminant species are less dependent on the quality of protein in feeds because bacteria present have the ability to synthesise high-quality proteins from non-protein or low quality protein sources (Huntington, 1986).

The success of the ruminal digestive system is dependent on the production of lysozymes. Lysozymes are bacteriolytic enzymes present in virtually all animals and

are normally found in macrophages, tears, saliva and mammalian milk (Jolles and Jolles, 1984). There are two major groups in the lysozyme *c* gene family: a conventional and a calcium–binding type; these appear to have arisen from an ancient gene duplication preceding the divergence of birds and mammals some 300 million years ago (Kornegay et al., 1994). The change in the regulation of these lysozymes occurred such that they were expressed in and became adapted to the stomach, becoming a digestive enzyme for ruminant species (Dobson et al., 1984; Jolles et al., 1984; Irwin and Wilson, 1990). Stomach lysozymes are derived from the conventional antibacterial lysozymes already present in mammals (Irwin and Wilson, 1990). This adaptation allowed the lysis and digestion of bacteria as they pass through the gut, preventing the loss of valuable nutrients assimilated by the bacteria (Kornegay et al., 1994). The ruminant lysozymes illustrate the important role of regulatory evolution in the adaptation of species to a new digestive function (Irwin and Wilson, 1990).

2.1.2 Anatomy of the Ruminant Digestive Tract

The development of the ruminant digestive tract begins in the very early stages of embryonic growth. The individual digestive organs develop at different rates, with respect to each other, and to total body growth during foetal and post-natal development. Even though the forestomach (rumen, reticulum and omasum) has the capability of rapid growth and metabolic development, the ruminant is born as a single stomached animal lacking the development and function of the forestomach compartments (Church, 1993).

Due to underdeveloped reticulum, rumen and omasum in the new-born ruminant, the act of suckling initiates a reflex contraction, closing the reticular groove and forming a tube from the oesophagus straight through to the omasum; this allows the milk to quickly pass to the abomasum, bypassing the rumen (Orskov, 1972). The newborn ruminant is unable to digest and utilise carbohydrates, with the exception of glucose and lactose, the principal carbohydrates in milk. The casein in milk clots due to rennin and the acidic environment of the abomasum, delaying the onward passage of milk to allow preliminary digestion (Holmes et al., 2003).

The digestive tract develops quickly in the calf and, by three weeks of age, pasture or feed pass directly into the reticulo–rumen, which enlarges and develops a population of microorganisms so that by seven to eight week of age the calf is able to digest feeds as efficiently as an adult cow (Holmes et al., 2003).

The stomach is divided into four main parts: rumen, reticulum, omasum and abomasum, and through a process of consumption, regurgitation, remastication and absorption, feed passes through the digestive tract (Figure 2.1).

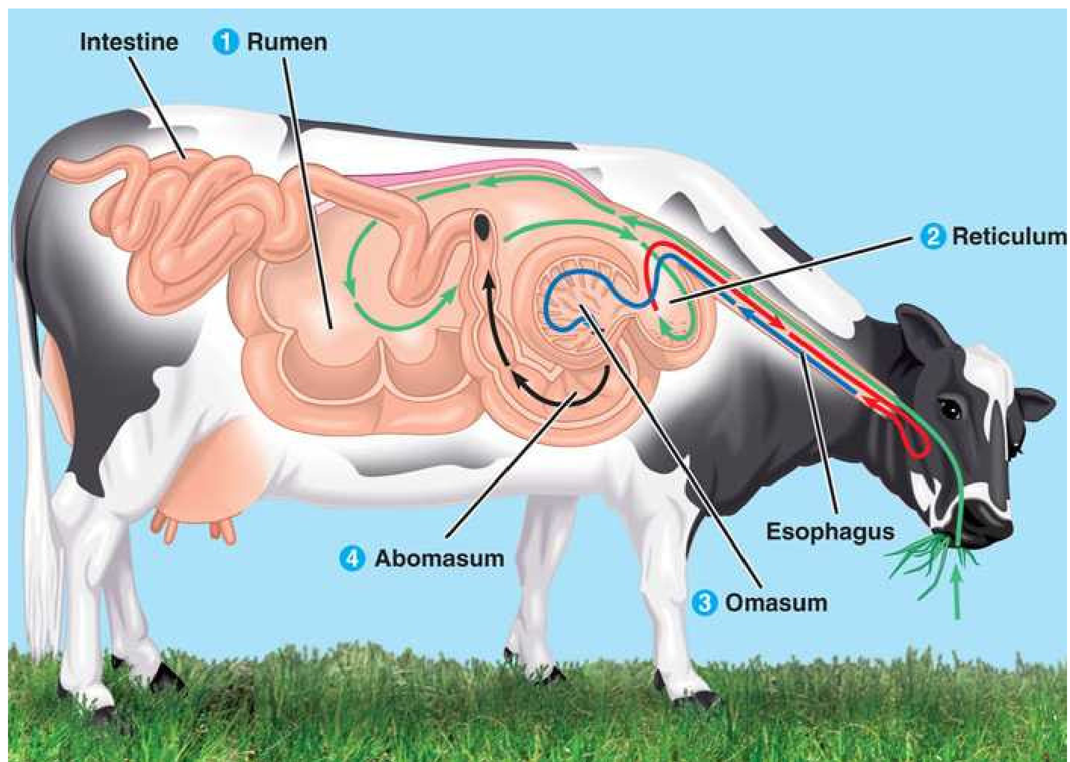


Figure 2.1 Diagram of ruminant digestive system illustrating the movement of feed. Green indicates feed eaten, red indicates regurgitated bolus (ruminating) back into the mouth, blue indicates the re-masticated bolus back into the digestive system and black indicates passage into small intestine.

Source: http://animalcareplc.blogspot.com.au/2012_11_01_archive.html

2.1.2.1 Reticulo-rumen

Due to their function and anatomical relationship, the reticulum and rumen are often collectively called the reticulo-rumen (Church, 1993). The epithelium of the reticulum is raised forming crests that intersect to form a 'honeycomb' appearance and are studded with small papillae (Habel, 1975). It is situated underneath and toward the front of the rumen allowing for the free movement of digesta between them. The reticulum walls do not secrete enzymes; instead, they function in moving ingested feed into the rumen, initiating regurgitation during rumination (Frandson et al., 2006). The reticulum is also a storage area for heavy foreign objects that may be eaten. The reticulum is partially separated from the rumen by the reticular fold, allowing for the mixing between the two compartments. This mixing recirculates undigested feed and aids in distributing microbes throughout the undigested feed (Church, 1993).

The rumen is the largest of all the stomach compartments, extends from the diaphragm to pelvis, and almost entirely fills the left side of the abdominal cavity. The rumen has visible grooves on the exterior that subdivide internal compartments by muscular pillars into the dorsal, ventral, caudodorsal and caudoventral sacs (Figure 2.2). The dorsal sac is the largest, and is continuous cranially with the reticulum over the reticular fold, allowing the two compartments to share a dorsal space (Frandson et al., 2006). The mucous membrane lining the rumen is non-glandular stratified epithelium, and is covered in small tongue-like structures called papillae that can be up to 12 millimetres (**mm**) long; these serve to increase the surface area for microbial growth and increase absorption of nutrients (Church, 1993). Separating the rumen from the omasum is the reticulo-omasal orifice that retains feed particles within the rumen until they are reduced to 1 to 2 mm in diameter (Domingue et al., 1991). The breakdown of feed particles is dependent upon the extent of chewing, rumination, fermentation, and the physical breakdown through mixing (Allen, 1996).

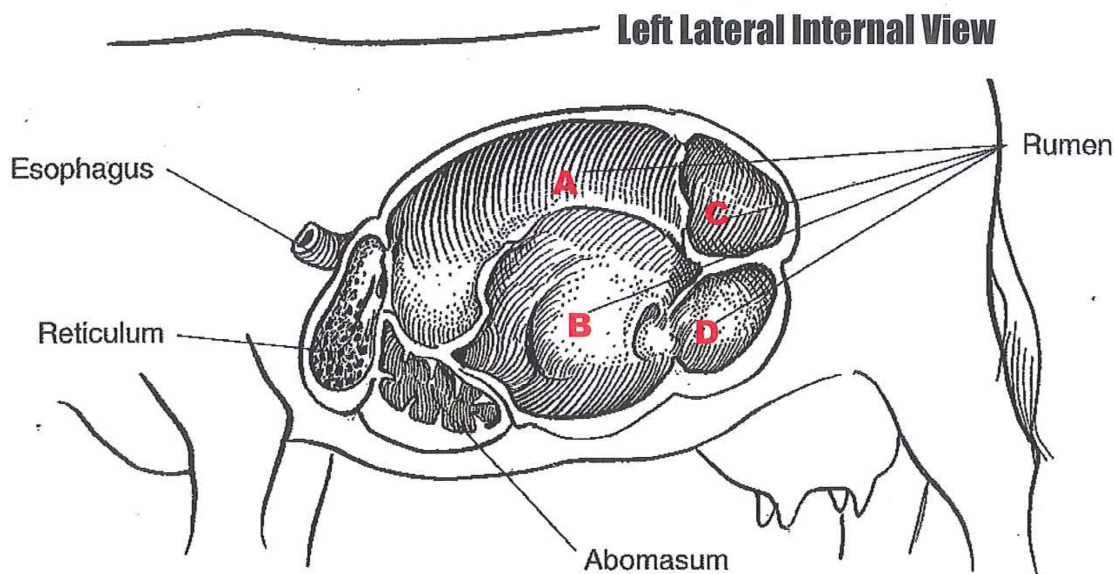


Figure 2.2 Diagram of internal view of the rumen indicating the subdivision into the (a) dorsal, (b) ventral, (c) caudodorsal, and (d) caudoventral.

Source: Frandson et al. (2006).

2.1.2.2 Omasum

The omasum is the third part of the stomach through which digesta passes. It is spherical in shape and is connected to the reticulum by a short tunnel; it is the smallest of the four stomach compartments (Frandson et al., 2006). The inside walls are covered in muscular laminae that lie in sheets, like the pages of a book (Stevens et al., 1960). The mucous membrane lining the lamina are studded with short, blunt papillae that grind digesta as it enters via the reticulo-omasal orifice before passing into the abomasum. The omasum functions, primarily, in the removal of excess water (Bost, 1970), which is demonstrated in the camel as it has a redundant omasum (Maloiy and Clemens, 1980), and the further reduction of particle size prior to entering the abomasum (Luginbuhl, 1983; Church, 1993); there is no secretion of enzymes. The omasum is connected to the reticulum and abomasum via the reticulo-rumen orifice and

omaso-abomasal orifice, respectively. The omaso-abomasal orifice is flanked on either side by folds of mucous membrane, the vela abomasica, which act as a valve to prevent the return of material from the abomasum to the omasum (Stevens et al., 1960; Frandson et al., 2006).

2.1.2.3 Abomasum

The abomasum, or 'true stomach', is the final compartment of the ruminant stomach is the first glandular region of the ruminant digestive tract and is structurally and functionally very similar to the stomach of monogastric animals (Frandson et al., 2006). Acid digestion, rather than microbial fermentation, takes place in the abomasum (Low, 1990), as in the monogastric animal. The epithelium of the abomasum consists of two main glandular regions, the fundic gland (region of the gastric glands) and the pyloric gland regions, and a smaller region containing cardiac glands. Fundic glands are simple tubular glands that open into gastric pits, where gastric secretions, such as hydrochloric acid and pepsins, are released (Low, 1990). The cells in the abomasum also secrete an intrinsic factor that is important for the absorption of vitamin B_{12}, which occurs in the small intestine (Elliot, 1980). The pH of the digesta entering the abomasum is around 6 but is quickly lowered to a pH of 1.0 – 2.5 by gastric secretions (Ash, 1961). The low pH is required for pepsin activity as above pH 4 activity ceases or is supressed (Taylor, 1959). The low pH also kills the microorganisms present, allowing for pepsins (enzymes) to begin the hydrolysis of both the microbial and dietary protein (Low, 1990). The pyloric glands and cardiac glands function is to secrete mucus (Luginbuhl, 1983); the mucus together with the bicarbonate form a protective layer to prevent pepsin erosion of the mucosa (Allen and Garner, 1980; Flemstrom and Garner, 1982; Low, 1990).

2.1.2.4 Small and Large Intestine

The small intestine is an elongated tube, made up of three regions: duodenum, jejunum and ileum. Partially digested feed pass from the duodenum and along the small intestine by peristaltic muscle contractions that start at the point where the abomasum

joins the duodenum. As digesta enter the small intestine, it mixes with secretions from the pancreas and liver. The small intestine is a major site for absorption of nutrients (Luginbuhl, 1983).

Secretions from the liver and pancreas enter the duodenum via ducts. The liver secretes bile to aid in the digestion of fats, whereas, pancreatic secretions are rich in enzymes and bicarbonate (Low, 1990). Enzymes secreted into the small intestine reduce any remaining proteins, starch and fat that escaped degradation, in the abomasum, into amino acids, glucose and fatty acids, respectively. As in the abomasum, the pH is vital for enzyme function; a low pH inactivates many of the duodenum-derived digestive enzymes. As digesta enter the duodenum, the pH is increased from 2.5 to 7- 8 by secretions from the liver and pancreas (Fallingborg, 1999).

The jejunum is lined with 'finger like' projections called villi, which are, themselves, lined with micro-villi that increase the surface area to improve the absorption of carbohydrates and protein (Trautman and Fiebiger, 1952). The main function of the ileum is the absorption of vitamin B_{12}, bile salts and any nutrients that were not absorbed by the jejunum (Drapanas et al., 1963). At the point where the ileum joins the large intestine, there is a valve, called the ileoceacal valve, which prevents digesta flowing back into the small intestine (Luginbuhl, 1983).

The large intestine consists of the caecum, which is a blind sac that forms the beginning of the large intestine, and the colon, which consists of ascending, transverse and descending parts. The descending colon ends at the rectum and anal canal. The caecum is a pouch connected to the large intestine and the ileum, and is separated by the ileoceacal valve (Luginbuhl, 1983). In herbivores, the caecum is enlarged and serves as a storage organ that allows bacteria and other microbes time to digest cellulose (Cork, 1996). Much of the large intestine is comprised of the colon, which is wider than the small intestine. The function of the colon is the active transport of sodium and the absorption of water by osmosis from the digesta (Luginbuhl, 1983). The bacteria present produce vitamins, such as vitamin K, thiamine and riboflavin, which are essential for the health and growth of the animal (Harmeyer and Kollenkirchen, 1989).

Undigested and unabsorbed food leaves the intestine in the form of faeces, via the rectum and anus.

2.1.3 Unique Aspects of Digestion in the Ruminant

2.1.3.1 Reticulo-rumen Motility

A pattern of reticulo-ruminal motility is initiated early in life, and persists for the lifetime of the animal (Holmes et al., 2003). These movements mix digesta, aid in the removal of gas, and move fluid and fermented feed into the omasum. Rumen motility is affected by diet. Long fibre particles increase distension and motility by stimulation of the mechanoreceptors in the reticulum and cranial sac (Leek, 1969). In comparison, rumen motility was decreased when unsaturated long chain fatty acids (**LCFA**) (Nicholson and Omer, 1983) and casein (Kil and Froetschel, 1994) were infused into sheep and steers, respectively. Reticulo-rumen contractions are dependent on bursts of nerve impulses in the vagal nerve and are essential to the maintenance of fermentation as a continuous process (Church, 1993). They are classified as either primary contractions (the mixing cycle) or secondary contractions (eructative contractions).

2.1.3.1.1 Primary Contractions

The primary contractions start in the reticulum, causing the reticulum and the reticulo-ruminal fold to contract to half its resting size. A more powerful second contraction follows, that passes caudally over the rumen, causing the cranial sac to lift, due to the contraction of the cranial pillars, and compression of the dorsal sac (Reid and Cornwall, 1959). The wave of contraction continues over the caudodorsal blind sac, ventral sac and caudoventral blind sac. The wave of contraction is followed by a wave of relaxation; so, when parts of the rumen are contracting, other parts are dilating. Wyburn (1980) illustrated the cyclic activity as a flow of digesta from the reticulum to the cranial sac, into the dorsal sac, and back through the cranial sac to the reticulum or into the ventral sac. A cycle of contractions occur every 50 to 70 seconds (i.e. 1,400

times a day), with the highest frequency recorded during feeding and the lowest when the animal is resting (Wyburn, 1980).

2.1.3.1.2 Secondary Contractions

The secondary contractions generally occur during the eructation process (removal of gases) and involve the dorsal coronary pillar, contractions of caudodorsal blind sac and dorsal sac, and relaxation of the caudoventral blind sac (Wyburn, 1980). The wave of contraction is in a circular motion to the dorsal blind sac, dorsal sac and ventral sac, and back to the ventral blind sac, and takes about 30 seconds to complete during the eructation process (Wyburn, 1980).

2.1.3.2 Rumination

Rumination is a vital process for ruminant animals, allowing for the rapid ingestion of feed and the completion of mastication later. Rumination is important in several ways:

1. It breaks down the particle size of the feed, allowing for passage from the rumen into omasum (Pearce and Moir, 1964).
2. It breaks down impermeable plant tissue, increasing the surface area available to the microorganisms (Gordon, 1968).

This ’double digestion’ system allows ruminants to physically break down the feed and to ferment cellulose, a widely available energy source that is otherwise indigestible (Baumgardt, 1969).

After a period of grazing, the rumination process begins, initiated with a reticular contraction that is distinct from the primary ruminal contraction. An extra-reticular contraction a few seconds prior to the usual ruminal contraction drops the intra-pleural pressure, due to contraction of diaphragm, allowing the movement of pre-ingested feed into the oesophagus and back up to the mouth (Church, 1993). The cow then re-chews the regurgitated matter, commonly called ‘chew the cud’, and then swallow. Studies have indicated that on grass-based diets, about twice as much dry

matter passes through a rumination cycle than is consumed; in comparison, on pelleted or ground diets rumination is absent or reduced (Ulyatt et al., 1984).

Ruminant species secrete large volumes of saliva, making up 70% of the fluid entering the reticulo-rumen. For example, a cow that ruminates for 6-8 hours (**h**) a day can produce over 250 litres of saliva (Wales and Doyle, 2003). Saliva also influences feed and water intake and rate of feed passage from the rumen (Bartley, 1976). The rumination process is continuous (i.e. every 1-2 minute; **min**) until the digesta is small enough to pass through the reticulo-omasal orifice to the omasum (Domingue et al., 1991).

2.1.3.3 Microorganisms

The rumen houses a diverse population of bacteria, protozoa and fungi, which digest feeds ingested by the cow. During fermentation, these microorganisms produce end-products that are utilised by the cow as well as the microorganisms, themselves, for their own reproduction and cell growth. The rumen environment is anaerobic; consequently, the inhabiting microorganisms are strict anaerobes and sensitive to oxygen (Russell and Hespell, 1981). The micro flora inhabiting the rumen is dense and contains approximately 10^{10} to 10^{11} bacterial and 10^{6} protozoal cells per millilitre (**ml**; Russell and Hespell, 1981). Diversity within this population is extensive, and over 200 species of bacteria, 100 species of protozoa and eight species of fungi have been described (McAllister and Cheng, 1996), although some of the isolates may be 'casual passengers' brought in with the food (Hastings, 1944). Microorganisms are either highly specialised, intermediate or very broad in the type of nutrients they use (Hungate, 1966a); for example, some digest starch and sugar, while others digest cellulose. Microorganisms that overlap in their ability to utilise a particular substrate increase the efficiency with which that substrate will be used (Bryant and Small, 1955). For constant microbial turnover, their generation interval needs to be shorter than the passage rate of rumen digesta, as the microorganisms lack the ability to store nutrients during times of abundance for use when nutrient supply is limited (Russell and Hespell, 1981; Wells and Russell, 1996).

2.1.3.3.1 Fungi

Fungi have been isolated from the rumen of numerous species, including sheep, goats, cattle and deer (Bauchop, 1981). Fungi can account for up to 8% of the microbial population (Orpin, 1983), but only a few of these produce highly active cellulases and hemicellulases to break down cellulose and hemicellulose (Trinci et al., 1994). Fungi are the first organism in the rumen to invade and commence digesting the structural plant components, beginning from the inside, reducing the tensile strength of the components and increasing particle breakdown during rumination (Akin et al., 1983). The damage caused by fungi allows bacteria to colonise the plant material (Bauchop, 1979 a,b).

2.1.3.3.2 Protozoa

The diet of the animal determines the species of protozoa in the rumen (Leng, 1982); for example, protozoa populations are low on fibrous diets; in comparison, their populations increase on diets high in starch and sugars (Leng, 1982). Protozoa are preferentially retained in the rumen (Minor et al., 1977; Bird and Leng, 1978) and isotope studies indicate substantial lysis in the rumen (Leng, 1982). Some protozoa are cellulolytic, but their major substrates appear to be micro-organism sugar and starches, which are rapidly assimilated and used for energy in their growth and maintenance (Dijkstra and Tamminga, 1995). Due to their larger cell volumes, protozoa are less metabolically active than bacteria (Russell and Hespell, 1981). Their fermentation products include acetate, butyrate, lactate, CO_2 and hydrogen. Besides contributing to volatile fatty acid (**VFA**) production, protozoa aid in sequestering carbohydrates from rapid bacterial attack and, thus, preventing the rapid fermentation to lactate that would lower the ruminal pH (Russell and Hespell, 1981).

2.1.3.3.3 Bacteria

Bacteria are normally the largest microbial biomass in the rumen, and they reside in one of three interconnecting environments within the rumen (Czerkawski, 1986):

1. The liquid phase, where bacteria in the rumen fluid feed on soluble carbohydrates and protein. These constitute up to 25 % of microbial mass.
2. The solid phase, where microorganisms are associated with or attach to food particles, digesting insoluble polysaccharides, such as starch, cellulose and hemicellulose, as well as the less soluble proteins. These constitute up to 70 % of the microbial mass.
3. Microbes that are attached to the rumen epithelium or to protozoa.

Ruminal cellulolytic microorganisms are pivotal in the nutrition of ruminant species on a forage diet, as cellulose is the most abundant component of plant cell walls (Weimer, 1996). Although a large number of bacteria, fungi and protozoa digest cellulose (Hungate, 1966b; Dehority, 1991; Weimer, 1992), the major cellulolytic bacteria *Ruminococcus albus*, *Ruminococcus flavefa*ciens, and *Fibrobacter succinogenes* account for 0.3 – 4% of the bacterial population (Krause et al., 1999; Weimer et al., 1999) and produce large quantities of acetate (Church, 1993). These bacteria are reportedly sensitive to acidity (Stewart, 1977); therefore, when the pH drops below 6, referred to as the cellulolytic threshold (Williams et al., 2005a), these bacteria can be eliminated or their growth rate supressed, resulting in decreased cellulose digestibility (Orskov and Fraser, 1975; Mould et al., 1983). However, Hoover (1986) reported that cyclic falls in rumen pH to below the cellulolytic threshold may only cause a moderate, transient reduction in fibre digestion, if it was only for short periods of time. Williams et al. (2005a) reported that although rumen pH fluctuates during the day, with decreases during grazing, supplementation with grain did not increase or extend the time that rumen pH was below 6. Kolver and de Veth (2002) reported that a low mean ruminal pH (5.6-6.2) on fresh pasture was associated with increased flow of microbial nitrogen from the rumen, increased VFA concentrations, increased DMI, and increased yields of milk, milk protein and milk fat, indicating that the performance of cows fed high quality pasture was not limited by a low rumen pH.

2.1.4 Rumen Fermentation

2.1.4.1 Carbohydrates

The energy metabolism of ruminant species is dependent on their ability to digest carbohydrates in their feed (Schofield, 2000). Carbohydrates come in the form of cellulose, hemicellulose and fructosan from grasses and forages, starch from cereal grains, and sucrose from root crops (Holmes et al., 2003). The most important end products of carbohydrate breakdown from microbial fermentation in the rumen is VFA (Bergman, 1990), contributing 70% of energy for the animal (Carroll and Hungate, 1954). The three major VFA produced are acetate (acetic acid), propionate (propionic acid) and butyrate (butyric acid; Sutton et al., 2003), which are absorbed across the rumen wall into the portal system and transported to the liver (Figure 2.3; Kristensen et al., 2000). The ratio of VFA produced depends on the type of feed being digested (Bergman, 1990; Carro et al., 2000). Diets comprised of forages tend to yield greater amounts of acetate and butyrate on a molar basis; for example, 65-72% acetate 15-25% butyrate and 8-15% propionate (Mackle et al., 1996; AFRC, 1998). Increasing the amount of starch and soluble carbohydrate increases the production of propionate (Murphy et al., 1982). Propionate and butyrate concentrations increase gradually in grazing cows, as the day progresses, with an increase from dusk reaching a peak just before midnight (Taweel et al., 2004).

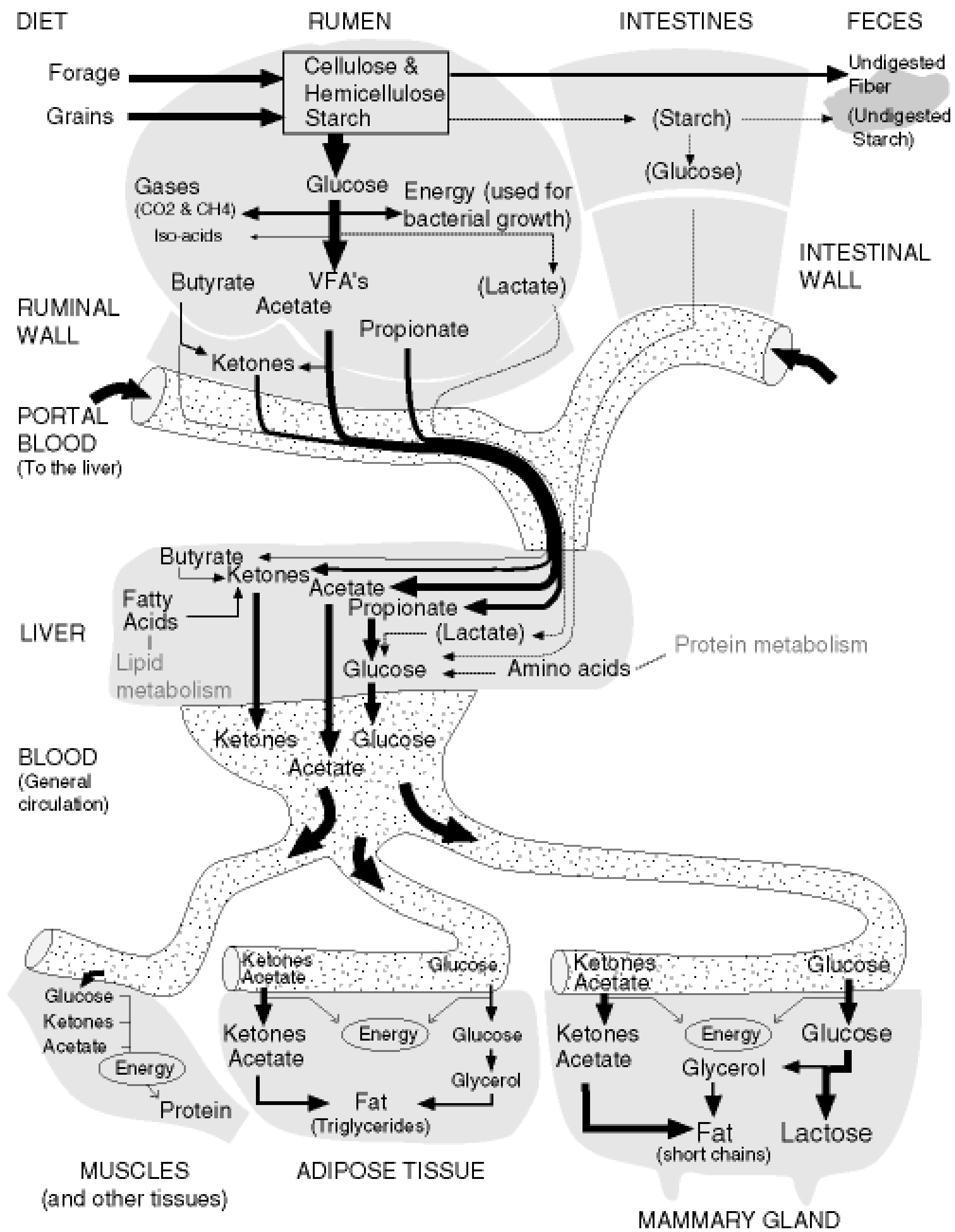


Figure 2.3 Overview of carbohydrate metabolism in the dairy cow.

Source: www.babcock.wisc.edu

2.1.4.1.1 Acetate

Acetate is the most important VFA produced in ruminant species and is an end product from the fermentation of fibre (i.e. cellulose and hemi-cellulose; Brockman, 2005). In a high forage diet acetate is the major VFA produced (Cronje et al., 1991). About 70% of acetate produced in the rumen can be accounted for in portal blood (Bergman and Wolff, 1971). Studies have concluded that the remaining 30% is utilised within the rumen, and are not used for rumen epithelial metabolism (Kristensen, 2001). Acetate is metabolised rapidly by the body and provides much of the energy requirements of ruminant tissues (Annison and Lindsay, 1961; Sabine and Connor Johnson, 1964). Acetate is the main precursor for lipogenesis in ruminants (Annison and Lindsay, 1961), due to the absence of adequate levels of adenosine triphosphate (**ATP**) citrate lyase (Hanson and Ballard, 1967); ATP citrate lyase is an enzyme that serves as a link between metabolism of carbohydrates and the production of fatty acids. Once absorbed from the blood, most acetate enters the tricarboxylic acid cycle (**TCA**) cycle (i.e. is oxidised) or used for fatty acid synthesis (Annison and Lindsay, 1961). Compared with the rat, activity of acetyl-Coenzyme A (**CoA**) synthetase is 2-3 times higher in ruminant adipose tissue, indicating the high rate of acetate to fatty acid synthesis in ruminant species (Hanson and Ballard, 1967).

2.1.4.1.2 Propionate

Propionate is an end product of the fermentation of starch and sugars, and is the major glycogenic substrate in fed ruminants (Danfaer, 1994), accounting for as much as 80% of glucose produced in lactating cows (Steinhour and Bauman, 1988). Propionate is converted to glucose or oxidised in the TCA cycle (Steinhour and Bauman, 1988) as well as stimulating oxidation of acetyl CoA derived from other fuels (Allen, 2000). Feeds high in rapidly fermentable carbohydrates, such as cereal grains, result in populations of bacteria that produce relatively more propionate and butyrate than acetate (Kristensen, 2005); rumen pH can be a contributing factor for this, as a lower pH can supress the activity of microorganisms that digest fibre (Figure 2.4). Propionate is considered a more efficient energy source because fermentation that favours production

of propionate produces less methane and CO_2 (Church, 1993; Moss et al., 2000). During absorption through the rumen epithelium, 2-5% of propionate is converted to lactic acid with the reminder entering portal blood as propionate (Church, 1993).

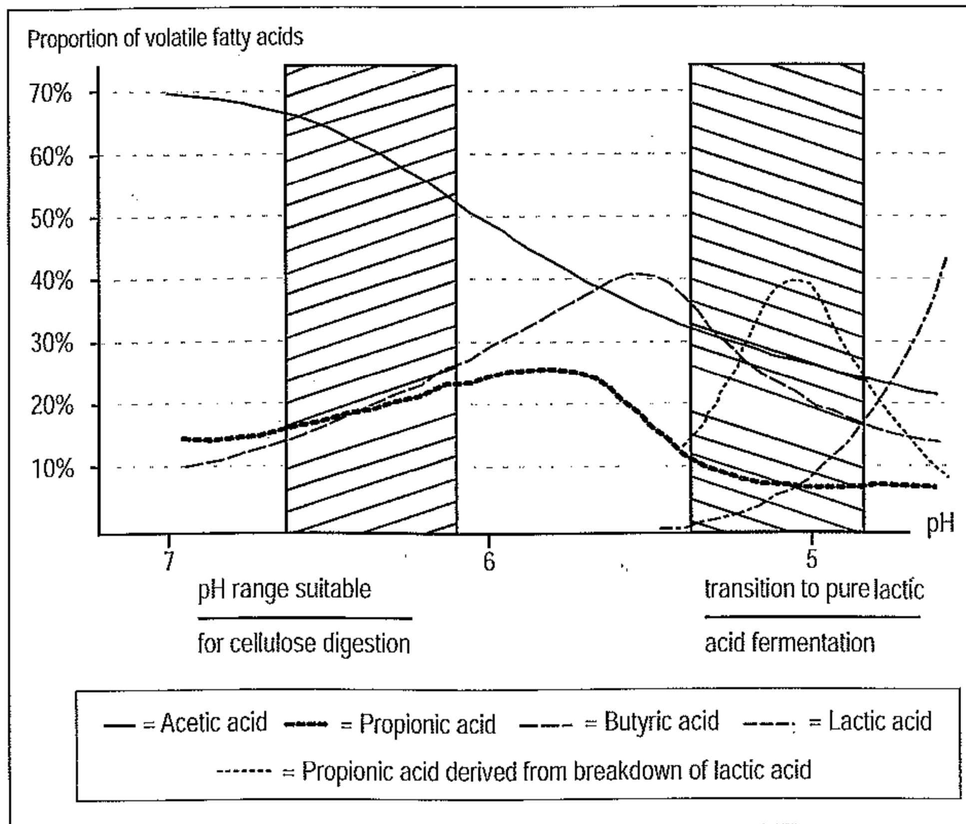


Figure 2.4 Proportions of VFA produced in the rumen when pH falls from 7.0.

Reprinted from Rosenberger, Clinical Examination of Cattle, 1979, Paul Parcey Scientific Publishers, Berlin and Hamburg.

2.1.4.1.3 Butyrate

Butyrate differs from acetate and propionate in that the amount of butyrate absorbed into the portal circulation is low in relation to the amount produced (Masson and Phillipson, 1951). This is due to butyrate being converted to "ketone bodies" during absorption across the rumen epithelium, mainly as β-hydroxybutyrate (**BHBA**), with the remainder as acetoacetate and acetone, (Pennington, 1952; Heitmann et al.,

1987; Reynolds et al., 1988). Any butyrate reaching the liver is rapidly metabolised by hepatic tissue (Reynolds et al., 1988). Butyrate is the VFA most favoured as an energy source for rumen epithelial cells (Pennington, 1952; Bugaut, 1987). The reason gut epithelia metabolise butyrate is not only to generate acetyl-CoA (Kristensen and Harmon, 2004), which is a common intermediate in the metabolism of acetate (Black et al., 1961), but also to decrease the butyrate load on the liver and peripheral tissue, as studies indicate it is toxic to the animal (Manns and Boda, 1967).

2.1.4.1.4 Other small VFA

Other smaller VFA are produced in the rumen, but in smaller quantities (Ishler et al., 1996). Valeric acid is produced as a result of protein deamination in the rumen (Shazly, 1952) and increases in production within one hour after feeding commences (Stewart et al., 1958). Small amounts of butyrate and valeric acid isomers (i.e. iso-butyric and iso-valeric) occur as products of the amino-acid metabolism of a number of anaerobes of the *Clostridium* genus (Cohen-Bazire et al., 1948).

2.1.5 Protein

In contrast to the non-ruminant, who must fulfil their protein requirements from dietary sources, dietary protein is of little importance in ruminant species as the rumen microorganisms are capable of synthesising their protein requirements (Huntigton, 1986; Kim et al., 2009; Figure 2.5). The amino acids produced from microbial protein represent 50 – 90% of the total protein absorbed in the small intestine (Jouany et al., 1998).

Rumen microorganisms break down rumen degradable protein (**RDP**) to amino acids and ammonia, which is a major source of nitrogen for microbial growth (Moran, 2005b). The rate of microbial growth is influenced by degradation of ruminal carbohydrates (Pathak, 2008). Therefore, the rate of carbohydrate digestion influences microbial growth and, hence, microbial protein (Hoover and Stokes, 1991). For

example, microbial protein synthesis is low in low quality forages due to slow carbohydrate degradation (Pathak, 2008).

If energy is limited, microorganisms become less efficient at using ammonia (Russell and Strobel, 1987). In such cases, surplus ammonia is absorbed across the rumen wall instead of being converted to microbial protein (Russell and Strobel, 1987). In the liver, ammonia is converted to urea, where it is either recycled to the rumen as non-protein nitrogen (**NPN**) in the saliva, which is then converted back to ammonia by the microorganisms or excreted in urine (Coleman and Barth, 1977).

Dietary protein that is directly available to the cow is referred to as undegradable dietary protein (**UDP**) and is hydrolysed and absorbed in the abomasum and small intestine, along with any RDP that has escaped microbial digestion (Church, 1993; Jouany et al., 1998). Undegradable protein and escaped metabolisable protein, provide a greater range of amino acids than protein broken down in the rumen (Moran, 2005b), which is restricted to the component amino acids assimilated in microbes (Ishler et al., 1996).

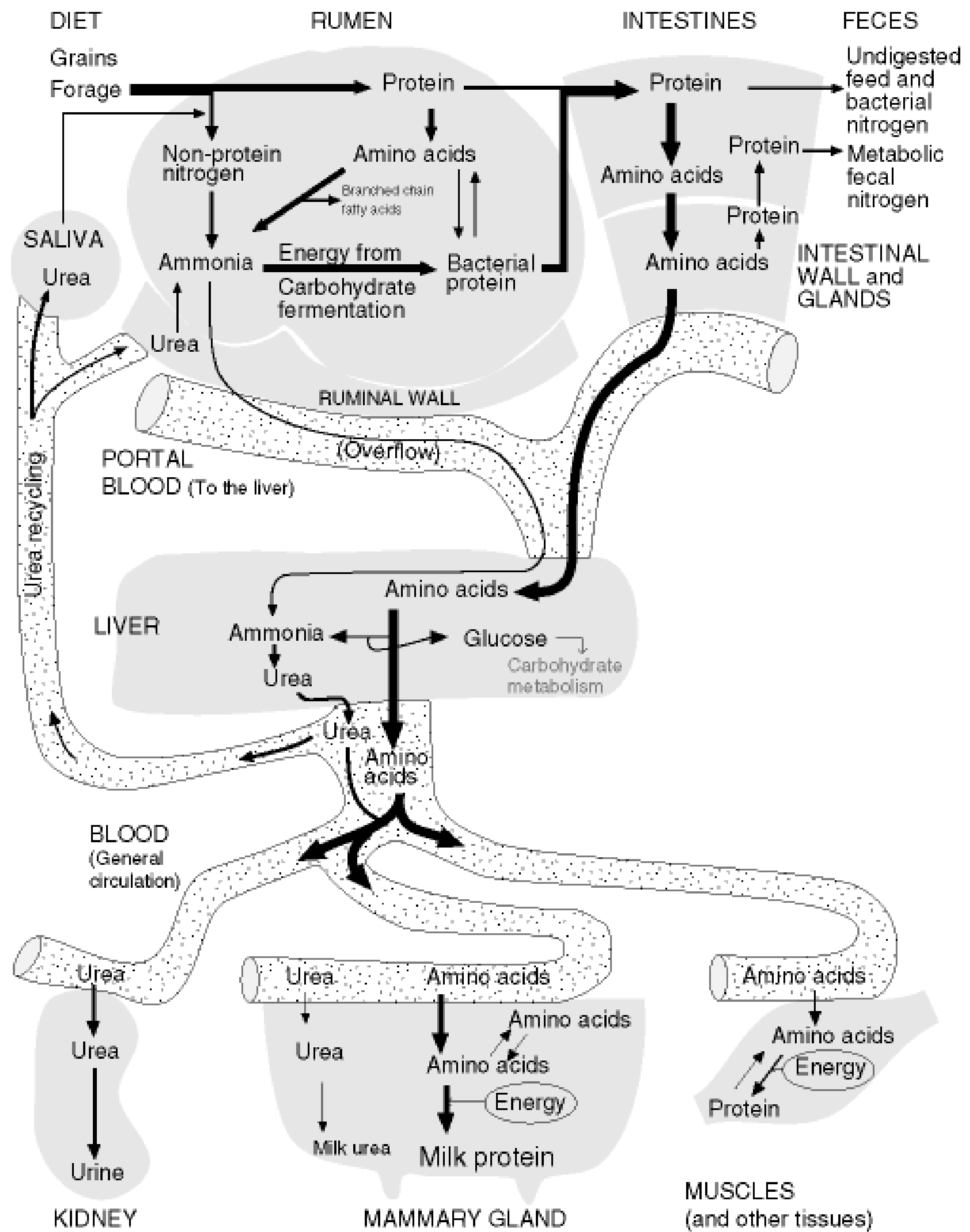


Figure 2.5 Overview of protein metabolism in the dairy cow.

Source: www.babcock.wisc.edu

2.1.6 Lipids

Lipid digestion in the ruminant begins in the rumen, compared with the small intestine in non-ruminants (Bauchart, 1993). Dietary lipids are usually triglycerides, phospholipids and galactolipids (Kim et al., 2009). Lipids are rapidly hydrolysed by bacterial lipases to free fatty acids, galactose and glycerol in the rumen (Figure 2.6; Jenkins, 1993). The major LCFA components of these are linolenic, linoleic and palmitic acids (Harfoot and Hazelwood, 1988; Kim et al., 2009). The carbohydrate constituents (i.e. glycerol and galactose) are converted to VFAs (mainly acetate and propionate; Garton et al., 1961), and although microorganisms cannot use fatty acids as an energy source, some fatty acids are used by bacteria for the synthesis of phospholipids, which are needed to build cell membranes (Bauchart, 1993; Ishler et al., 1996). Microbial phospholipids and LCFA are digested in the small intestine and contribute to the pool of fatty acids that are processed and absorbed through the intestinal wall (Bauchart, 1993). To prevent rumen fermentation problems, the total diet DM should contain less than 5% fat (Church, 1993). Beyond this level, fat will coat dietary fibre, resulting in an inability of microorganisms to attach to fibre and, thereby, reducing the ruminal digestion of structural carbohydrates (Ikwuegbu and Sutton, 1982).

Lipoproteins are aggregates of lipids and proteins that function to transport lipids around the body and facilitate lipid utilisation by emulsifying lipids to move through water, inside and outside cells. In farmed ruminant species, the composition and rate of secretion of lipoproteins are among the main factors that control lipid utillisation by tissues, including milk fat (Bauchart, 1993).

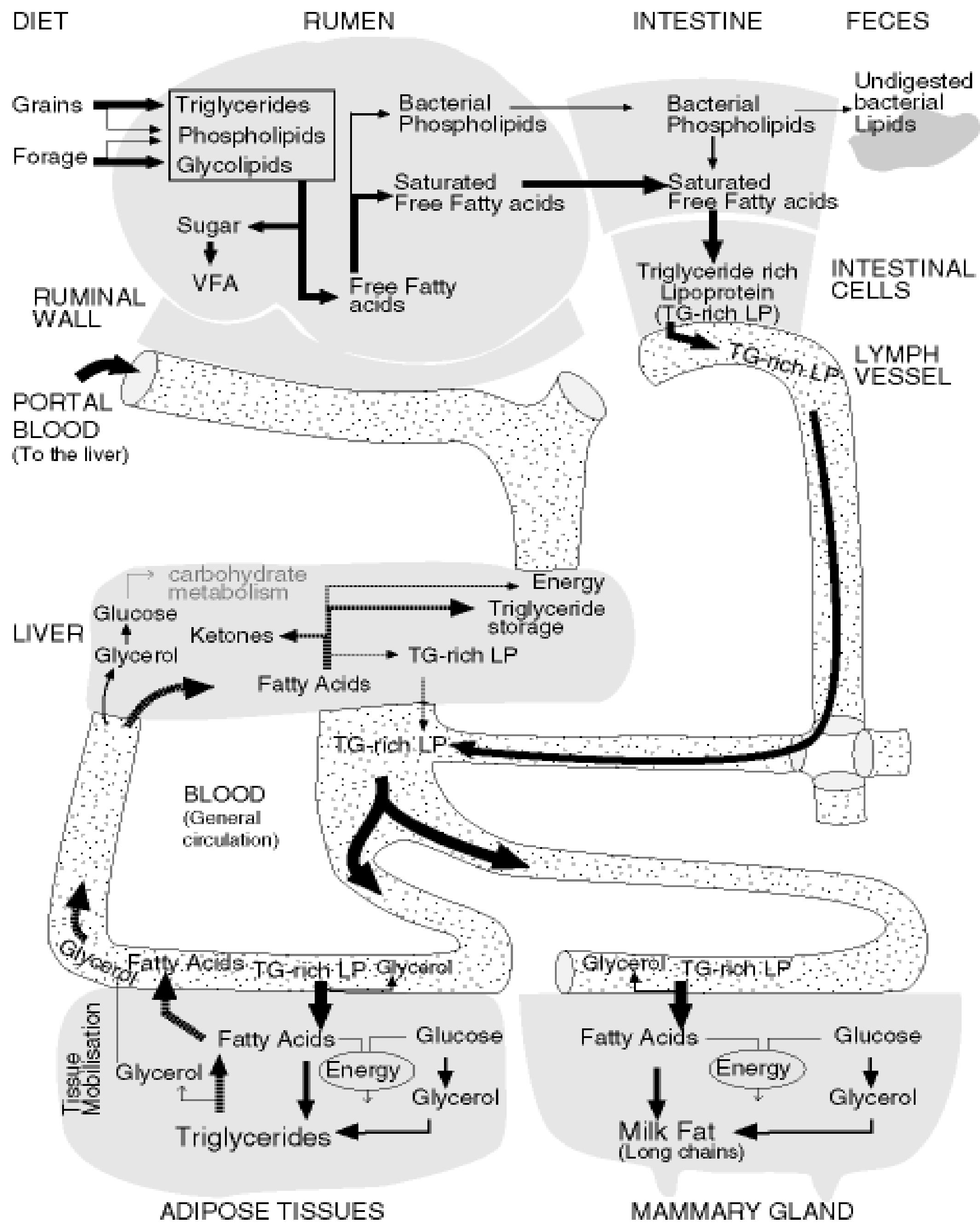


Figure 2.6 Overview of fat metabolism in the dairy cow.

Source: www.babcock.wisc.edu

2.1.6.1 Ruminal Absorption

Volatile fatty acids, which can be toxic to microbial metabolism (Russell and Hespell, 1981), are absorbed and metabolised by rumen epithelial cells into the portal system (Kristensen, 2005). The rate of VFA absorption is influenced by chain length (Ishler et al., 1996). Increasing the chain length increases the absorption rate, resulting in the following order of absorption: butyrate, propionate and acetate (Ishler et al., 1996). However, net absorption is dependent on the quantity metabolised by the rumen wall (Bergman and Wolff, 1971). Utilisation rates by the rumen wall are greater for butyrate, then propionate and followed by acetate (Bergman and Wolff, 1971); therefore, acetate will enter the blood in the greatest quantity relative to rumen production due to its low utilisation by the rumen wall.

Ruminant species differ from non-ruminants as large amounts of ketone bodies are released into the portal system due to the absorption and metabolism of butyrate in the rumen epithelium (Heitmann et al., 1987; Reynolds et al., 1988). Baird et al. (1975) reported that 12% of the non-lactating dairy cows' digestible energy is metabolised through ketone bodies. Ketone bodies can be used as oxidative substrates in heart (Williamson and Krebs, 1961), kidney (Weidemann and Krebs, 1969), skeletal muscle (Ruderman and Goodman, 1973) and the lactating mammary gland in both ruminants and non-ruminants (Heitmann et al., 1987). β-hydroxybutyrate is also used as a source of energy for fatty acid synthesis in adipose and mammary tissue (Ishler et al., 1996). The liver in fed ruminants always takes up acetoacetate at rates similar to alimentary release (Heitmann et al., 1987), but releases BHBA 3-fold higher in fed ewes (Heitmann et al., 1987), and 10- to 15 fold higher in lactating dairy cows (Baird et al., 1975; Baird, 1977) than alimentary release.

2.1.7 Gluconeogenesis

Glucose is the most important metabolic fuel for living organisms (Gomez-Ambrosi et al., 2009) and the glucose needs of ruminant species are similar to that post-absorptive in non-ruminant species (Brockman, 1978). However, intermediary glucose

metabolism differs, as the majority of dietary carbohydrate is fermented to VFA, compared with digestion of dietary carbohydrates and absorption of glucose in the non-ruminant (Brockman, 1978; Gomez-Ambrosi et al., 2009). Therefore, glucose requirements of ruminant animals are met by glucose synthesis from non-carbohydrate sources; this is termed gluconeogenesis (Brockman, 1978; Frandson et al., 2006).

In ruminant species, the major glucose precursors are propionate, lactate/pyruvate, amino acids and glycerol (Brockman, 1978). In the fed animal, absorbed propionate and amino acids are the major precursors (Brockman, 1978). The liver and kidneys are the only organs capable of significant gluconeogenesis, as they are the only organs that express the enzymes necessary for gluconeogenesis (Gomez-Ambrosi et al., 2009); of these organs, the liver contributes the majority (80%; Cryer, 2003).

The quantity of gluconeogenic precursors available to the liver is the major factor determining the amount of glucose formed. In ruminants, gluconeogenesis increases after feeding and decreases during fasting, due mainly to the amount of precursor available (Brockman, 1978). Whereas, in non-ruminant species maximum gluconeogenesis occurs only if a meal has not been eaten recently (Church, 1993). The rate of hepatic glucose production is regulated by the rate of glucose uptake by peripheral tissue (DeFronzo and Tripathy, 2009).

2.2 Grazing Behaviour

It has been suggested that grazing animals are not distinct meal eaters, as they can spend most of the day eating (Forbes, 2007). This is due to the slow rate at which they consume pasture to meet their requirements. When the rate of eating easily outpaces the rate of utilisation of nutrients (i.e. TMR), meals are discrete (Thorne et al., 2003; Forbes, 2007). However, regardless of the way feed is offered, ruminant species consume their feed in periods that alternate between rumination and idling (Forbes, 2007). Feeding is the predominant behaviour in ruminant species and is demonstrated when feeding behaviour takes priority over rumination, whenever the causal factors of the two activities conflict (Metz, 1975).

Grazing is predominately a daylight activity, with 65-100% of daily grazing taking place between 0600 and 1900 h over a wide range of environmental temperatures, supplementation regimes, grazing management protocols and pasture DMI (Krysl and Hess, 1993). A summary of reported time spent grazing daily is presented in Table 2.1. Daily grazing time consists of a cluster of discrete meals, or grazing bouts (Gibb et al., 1998); regardless of meal frequency, cows exhibit three main grazing bouts: sunrise, afternoon, and just prior to sunset (Gregorini et al., 2006). The intensity of grazing can change depending on the time of day, with the grazing bout prior to sunset characterised as the most intensive grazing bout of the day (Gibb et al., 1998; Taweel et al., 2004). The intensive pre-sunset grazing bout facilitates maximum rumen fill before darkness (Gibb et al., 1998), when very little grazing occurs (Gibb et al., 1998; Taweel et al., 2004).

Cows will graze when offered fresh pasture (Orr et al., 2001); however, the length of time spent grazing if fresh pasture is offered in the a.m. differs from fresh pasture allocation in the p.m. When fresh pasture is allocated in the morning, time spent grazing is in short grazing intervals, with an intensive grazing bout still evident in the later afternoon (Orr et al., 2001). In comparison when offered a fresh pasture allocation in the afternoon, cows exhibit a long uninterrupted grazing session (Orr et al., 2001).

The majority of ruminating occurs during the night, with ruminating intervals between grazing bouts during the day (Phillips and Leaver, 1986). Time spent in idle behaviour is necessary for rest and social interactions, but is also an essential element in the digestive process (Gibb, 2006). Idle behaviour allows feed particles, made buoyant by the production of gas in microbial digestion, to rise to form a fibrous mat, which is then regurgitated for re-mastication (Gibb, 2006). The choice of a particular grazing behaviour depends on the current state of the animal, its environment and possibly past and anticipated states (Mangel and Clark, 1986).

Table 2.1 Daily time spent grazing and ruminating (min) for North American (NA) and New Zealand (NZ) pasture-fed cows.

Reference	Time spent grazing (min)		Time spent Ruminating (min)	
	NA	NZ	NA	NZ
Linnane et al. (2004)	661	628	389	374
McCarthy et al. (2007b)	605	615	505	513
Thorne et al. (2003)	510	558	444	450
Taweel et al. (2004)	510	NR	NR	NR
Gibb et al. (1998)	566*	NR	434*	NR
Rook et al. (1994)	685	NR	301	NR
Rutter et al. (2002a)	536	NR	526	NR

NR = not recorded

* Average time from two separate trials within the same study.

2.2.1 Environment

Solar radiation directly or indirectly exerts a profound effect on behaviour (Hafez, 1969), whether in the form of thermal radiation, providing heat, or in the visible form, light, which provides the photoperiod effect that regulates many, if not all, diurnal and seasonal activity patterns (Hafez, 1969). The functional day begins 25 min before sunrise and ends 25 min after sunset (i.e. when the sun is about 6 º below the horizon; Hafez, 1969). The timing of pre-sunset grazing changes as day length changes during the year (i.e. evening grazing always starts 3-4 h before sunset regardless of time of sunset; Rutter et al, 2002b), even in high latitudes (e.g. the Netherlands) where sunset is around 2200-2300 h in summer (Taweel et al., 2004).

Rutter et al. (2002b) reported on the effects of a solar eclipse on grazing behaviour in a unique opportunity that arose in 1999. They reported that a total solar

eclipse starting at 10:11 Greenwich Mean Time (**GMT**) and lasting for 2 h 2 mins did not affect grazing or ruminating behaviour. The authors suggested that the period of light change from light to dark back to light was relatively short compared with decreasing light in the evening and that this may account for the lack of effect reported. Phases of the moon have also been reported to influence DMI, with almost complete suppression of night time grazing during a new moon (i.e. the dark phase; Gibb, 2006).

Heat stress, due to increased temperature, relative humidity and/or solar radiation, increases body temperature and respiration rate and can reduce DMI and milk production (Schütz et al., 2010). Grant and Albright (1995a) reported a 3-4 kg/day decrease in DMI in mid lactation dairy cows under heat stress. In hot temperatures, cows prefer to graze during the cooler mornings and evenings, and seek shade or spend time in idle or ruminating behaviour during the midday heat (Albright, 1993).

2.3 Intake Regulation in the Ruminant

Dry matter intake is fundamental to nutrition; it determines the level of nutrients ingested and, therefore, the animal's response and performance (Van Soest, 1994; Kolver and Muller, 1998; Roche et al., 2007b). Relatively low DM and ME intakes are primary limitations to productivity in pasture-based dairy systems, resulting in nutrient intakes that are insufficient to match the milk production potential of the grazing dairy cow (Kolver and Muller, 1998). Many factors affect DMI, with individual hypotheses based on physical fill of the reticulo-rumen (Mertens, 1994; Allen, 1996) or metabolic-feedback factors (Mertens, 1994; Illius and Jessop, 1996) proposed in the regulation of DMI, but it is most likely to be the additive effect of several stimuli (Forbes, 2007).

2.3.1 Physical Factors

2.3.1.1 Presentation of Feed

Pasture growth and composition is not constant; therefore, DMI is affected by grazing management (Holmes et al., 2003). When sward height (**SH**) is greater than optimum the proportion of stem and dead material in the sward increases compared with the more digestible leaf, and the quality of pasture declines (Stobbs, 1973; Baker et al., 1981). Conversely, as SH decreases below optimum, animal performance is compromised by reduced pasture DMI (Rook et al., 1994) because of a low DMI rate per unit of time spent grazing and constraints on the total time available through the day for grazing (Gibb et al., 1997). Rook et al (1994) reported a lower bite mass and rumination time in cows grazing swards of 4 centimetres (**cm**) compared with cows grazing 6 and 8 cm. Gibb et al. (1997) reported reduced DMI and greater rumination time in cows grazing a 9 cm sward compared with a 7 cm sward. Penning et al. (1991) suggested that the lower biting rate of sheep grazing tall swards was due to the greater bite mass increasing mastication and ruminating times. Parker and McCutcheon (1992) recommended ideal sward heights of 5–7 cm height during lactation to maximize production in both single- and twin-rearing ewes. Despite a high quality sward in low SH swards, increased grazing time and bite rate cannot compensate for the decrease in bite mass (Gekara et al., 2001).

Pasture allowance (**PA**) has been identified as one of the most important factors influencing pasture DMI in dairy cows (Hodgson and Brooks, 1999). Pasture allowance is measured as kg DM/cow per day (Tozer et al., 2004). There is a curvilinear relationship between pasture DMI and PA (Stockdale, 1985; Bargo et al., 2003), with pasture DMI increases as PA increases, but at a progressively declining rate (Taweel, 2006). Pasture DMI increased in high producing dairy cows when offered a higher daily PA (Maher et al., 1997; Dalley et al., 2001). Wales et al. (2001) reported an increase in pasture DMI of 0.7 kg after increasing daily PA by 4 kg DM in low merit milk producing cows. Similarly, Dillon and Buckley (1998) reported an increase of 0.5 kg DM after increasing daily PA by 3 kg in high merit milk producing cows. The

allocation of additional pasture to improve performance is therefore, debatable, due to small additional increases in daily DMI from further increases in daily pasture allowance (Stakelum, 1996).

Pasture utillisation is low if pasture is grazed insufficiently and this can lead to poor pasture quality in subsequent rotations (Stakelum, 1996). The speed of pasture digestion increases with increasing quality or digestibility. Digestibility of neutral detergent fibre (**NDF**) is an important parameter of forage quality (Allen and Oba, 1996). Forages with higher NDF digestibility allow greater DMI, most likely due to the reduced retention time in the rumen (Allen, 2000). Mertens (1987) suggested that DMI of dairy cows could be predicted by dietary NDF, in part because of the positive relationship between NDF and the bulk density of feeds. There are some exceptions, however; clover, with the same digestibility as grass, will ferment faster. This is due to the clover having greater proportion of cell contents (i.e. NSC, CP) and less cell wall constituents, resulting in a more rapid breakdown of cell walls, and a shorter retention time in the rumen (Wales et al., 2005); therefore, animals will eat more clover than grass (Ørskov, 1987). Consistent with this, Williams et al. (2005b) reported that for the same DMI, rumen fill was greater in animals eating perennial ryegrass compared with those eating clover. Cows eating ryegrass spent less time eating and more time ruminating than those eating clover. Dry matter intake is reduced as the feed ferments slower, due to particle size, remaining in the rumen until small enough to pass out of the rumen.

Some plants are less palatable to ruminants and this can be species specific (e.g. some plants eaten by cows will be rejected by sheep; Ørskov, 1987), and in some cases ruminants will demonstrate a preference for a particular cultivar if given a choice. For example, both sheep and cows are reported to prefer white clover in the morning and perennial ryegrass in the afternoon (Gibb, 2006). It was hypothesised that they did this to take advantage of the increase in water soluble carbohydrates in the ryegrass.

2.3.1.2 Supplementation

Feed supplements are offered to increase total DM and ME intakes (Stockdale, 2000b); however, incremental increases in supplementary feeds do not result in equivalent increases in total DMI (Mayne, 1991). This difference occurs because offering feed supplements reduces pasture DMI; this is known as substitution (Bargo et al., 2003), with the amount of pasture refused relative to supplement fed referred to as substitution rate (**SR**). Substitution rate is reflected in a reduction in grazing time (McGilloway and Mayne, 1996). Bargo et al. (2003) reported a 12 min decrease in grazing time for every 1 kg DM supplement consumed.

Animal responses to supplementary feeds vary due to the degree of SR (Stockdale, 2000a; Penno, 2002) and can be influenced by the type of supplement offered. Forage supplements decrease pasture DMI more than concentrate supplements at both low and high PA (Mayne and Wright, 1988; Stockdale, 2000b). Substitution rate ranged from 0.84 to 1.02 kg/kg for grass silage supplementation and from 0.11 to 0.50 kg/kg for concentrate supplementation (Mayne and Wright, 1988). Also, the type of carbohydrate supplemented is reported to affect SR. Meijs (1986) reported a reduction of SR from 0.45 kg pasture/kg high-starch concentrate to 0.21 kg pasture/kg fibre-based concentrate. Stakelum and Dillon (1988) reported increases in pasture DMI up to 1.5 kg DM per day when animals consumed 3 kg/d of high- fibre compared with high-starch supplement. In contrast to these data, however, Sporndly (1991) and Fisher et al. (1996) reported no effect of carbohydrate type on pasture DMI.

The greatest benefit to supplementing grazing cows is when pasture availability is low (McGilloway and Mayne, 1996). Meijs (1986) reported a low SR when PA was low. Stockdale, (2000a) reported similar levels of substitution, irrespective of supplement type (i.e. cereal grain or hay), when PA was low. Perez-Prieto et al. (2011) reported that supplementation decreased pasture DMI from 11.6 to 7.6 kg DM/d at low PA and from 13.1 to 7.3 kg DM/d at high PA, indicating a lower SR when PA is low. These data indicate that although supplementation decreases pasture DMI, the degree of

SR is determined by supplement type and pasture characteristics; with good pasture management practices, relatively large responses to supplements can be achieved.

2.3.1.3 Cow Genetic Merit

Successful grazing systems require dairy cows that are capable of achieving large pasture DMI relative to their genetic potential for milk production, meeting their nutritional requirements almost entirely from grazing (Dillon, 2006). Although increases in DMI are evident with increasing genetic merit for milk yield, they are small relative to the large differences in milk production (Veerkamp et al., 1994). For example, high genetic merit cows produced 30% more milk than low yielding cows, but only had a 6% greater DMI (Patterson et al., 1995). Differences in genetic merit in this case were largely determined by the ability of the cow to partition nutrients to milk yield rather than body tissue (Butler and Smith, 1989; Veerkamp et al., 1994; Horan et al., 2004; Roche et al., 2009).

North American (**NA**) Holstein Friesian (**HF**) cows have been selected for increased milk production when fed predominately TMR (Rauw et al., 1998). In contrast, the New Zealand (**NZ**) HF has been selected for milk production on a predominately pasture-based diet with little supplementation (Harris and Kolver, 2001). Horan et al. (2006) reported 0.4 kg DM/d difference in pasture DMI between high-producing NA HF and NZ HF cows. Linnane et al. (2004) reported no effect of strain on grazing time; however, high producing NA cows consumed more pasture DMI than their NZ counterparts, although they were unable to maintain body condition and live weight on a pasture-fed diet, indicating the unsuitable genetics for the NZ pasture system. Therefore, the choice of strain may depend on the feeding system (Linnane et al., 2004).

2.3.1.4 Physiological state

Energy balance is defined as the difference between energy intake and the energy required for maintenance, activity, milk production and pregnancy (Butler and

Smith, 1989; McNamara et al., 2003). The level of DMI varies according to stage of pregnancy, with DMI decreasing during the latter stage of gestation (Moran, 2005a) and very low DMI on the day of parturition (Marquardt et al., 1977; Roche, 2006). Olsson et al. (1998) reported a decreased DMI at calving time in all cows independent of the energy level in the diet. At parturition, pasture DMI is only about 50% to 70% of the maximum at peak DMI (Moran, 2005a). Following parturition, DMI needs to increase in order to meet the high nutrient demands of milk production (Roche et al., 2000).

During early lactation, maximum DMI follows peak milk production (Figure 2.7; Butler and Smith, 1989). Homeorhetic mechanisms ensure that body tissue, primarily adipose stores, is mobilised to support milk production (Butler and Smith, 1989; Remppis et al., 2011). Approximately 50-70 days post-calving (Roche et al., 2007a), cows change from negative to positive energy balance that results in a greater partitioning to body reserves rather than milk production.

The age and parity number of the cow can influence DMI as well as body condition. Primiparous cows eat less DMI than multiparous cows (Dado and Allen, 1994; Grant and Albright, 1995b) and have a slower rate of increase in DMI during the first five weeks of lactation (Kertz et al., 1991), while fat cows generally eat less than thin cows (Treacher et al., 1986; Roche et al., 2009). This is hypothesised to be a mechanism for regulating adipose tissue stores (Kennedy, 1953). It was suggested that the rapidity of fatty acid synthesis in adipose tissue of thin cows might reduce blood levels of lipogenic precursors, enhancing their absorption from the rumen and stimulating DMI (Bines and Morant, 1983).

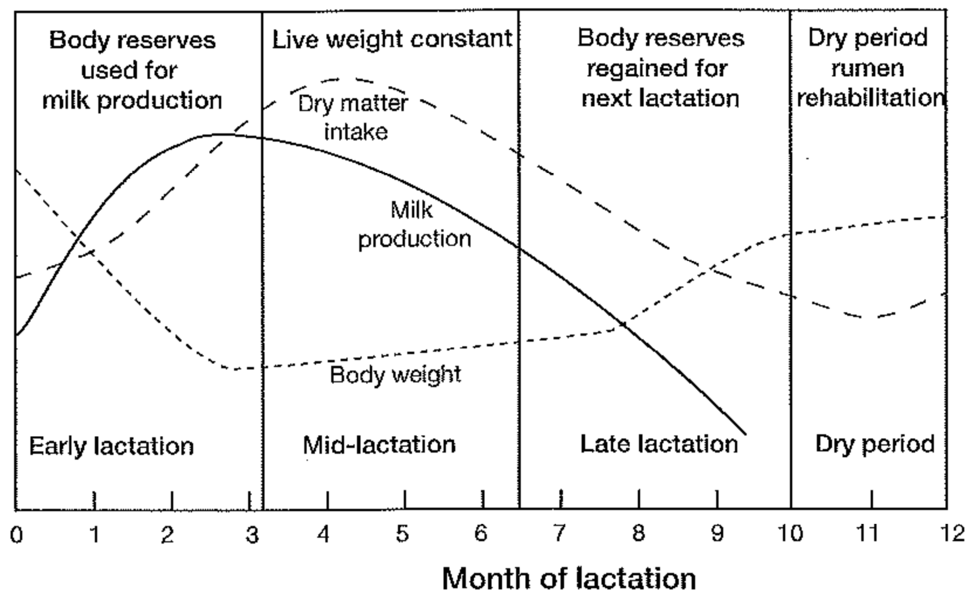


Figure 2.7 Dry matter intake, milk yield and live weight in a Friesian cow during the lactation cycle.

Source: www.landlinks.com.au

2.3.2 Physiological Factors

Feed intake regulation in non-ruminant species is based on the concept of hunger and satiety, where an animal eats until its metabolic requirements are met and excess circulating nutrients trigger the cessation of eating (NRC, 2001). Seoane et al. (1972) exchanged blood from a satiated sheep into a fasted sheep and vice versa and reported an increase in DMI in the satiated sheep and a decreased DMI in the fasted sheep. Although actual factors were not identified, this experiment highlighted that humoral factors influence DMI.

2.3.2.1 Hunger and Satiety

The terms hunger and appetite are often incorrectly used interchangeably. Hunger is the physiological "need" for food, while appetite is the "desire to eat" and is associated with sensory experiences or aspects of food, such as sight and smell of food, emotional cues and social situations. Hunger acts as a basic drive to eat, while appetite is more a reflection of eating experiences. At times people are not hungry but have an appetite, such as seeing dessert after eating a full meal, or may be hungry but have no appetite, such as when then are sick. Satiety is the physiological and psychological experience of "fullness" after eating and/or drinking. Satiety can be quantified by determining the duration between meals and/or the amount of energy consumed at the subsequent meal (Little et al., 2005).

To study the regulation of food intake, defined periods are studied; the fasted state, the period just after a meal, and the post-absorptive state. However, ruminant species differ from non-ruminant species in that there is a constant flow of digesta through the gastrointestinal (**GI**) tract, as opposed to discrete episodes of food passage associated with meals in the non-ruminant. Therefore, intake regulatory factors may differ across species. The key components involved in the physiological regulation of intake include the hypothalamus, nucleus solitary tract (**NTS**), gastrointestinal tract and associated organs and adipose tissue.

2.3.3 Anatomy of the Brain Pertaining to Intake Regulation

2.3.3.1 Blood Brain Barrier

Neurons within the central nervous system (**CNS**) communicate using a combination of chemical and electrical signals that are precisely regulated. The blood brain barrier (**BBB**) is one of three barriers at key interfaces between blood and neural tissue, which play a major role in this regulation (Abbott, 1992). The BBB is a selective diffusion barrier at the level of the cerebral micro-vascular endothelium, characterised by the presence of tight-cell junctions and a lack of fenestrations.

Functions of BBB:

- *Ion regulation:* keeps the ionic composition stable and optimal for synaptic signalling, via ions channels and transporters.
- *Neurotransmitters:* the central and peripheral nervous system use many neurotransmitters; the BBB helps to separate the central and peripheral pools, eliminating 'crosstalk'.
- *Macromolecules:* the BBB prevents many macromolecules from entering the brain; for example albumin, pro-thrombin and plasminogen, which are damaging to nervous tissue, causing seizures, scarring and cell death.
- *Neurotoxins:* protects the CNS from neurotoxic substances circulating in the blood.
- *Brain Nutrition:* the BBB has a low passive permeability (via specific transport systems) to many essential water-soluble nutrients and metabolites required by nervous tissue.

2.3.3.2 Median Eminence

The median eminence (**MeE**) is the structure at the base of the hypothalamus and is one of eight circumventricular organs (regions surrounding the cerebral ventricles) in the CNS. The BBB surrounding the MeE is fenestrated, enabling the MeE to sense and respond to chemical signals from the circulatory system. The MeE conveys signals between the hypothalamus and peripheral endocrine system through the hypophyseal portal system, which is the only portal system in the brain (Green and Harris, 1949). This integrated communication between the hypothalamic pathways and the MeE is essential for the regulation of energy homeostasis (Hillebrand et al., 2002a).

2.3.3.3 The Hypothalamus

Over the past 20 years, knowledge regarding the role of the hypothalamus in the regulation of feeding has substantially improved (Arora and Anubhuti, 2006). Anatomically, the hypothalamus is divided into three broad domains termed the posterior, tuberal and anterior regions (Table 2.2). Each of these regions are further

subdivided into medial and lateral areas. These defined areas, contain nuclei that interconnect neuronal circuits that respond to changes in energy status by altering the expression of specific neuropeptides (Flier, 2004; Morton et al., 2006; López et al., 2007).

Table 2.2 The defined regions, area and respective nuclei within the hypothalamus

Region	Area	Nuclei
Anterior	Medial	• Medial preoptic nucleus • Supraoptic nucleus • Paraventricular nucleus (PVN) • Suprachiasmatic nucleus • Anterior hypothalamic nucleus
	Lateral	• Lateral preoptic nucleus • Lateral nucleus (LT) • Part of supraoptic nucleus (SO)
Tuberal	Medial	• Dorsomedial hypothalamic nucleus (DMH) • Ventromedial nucleus (VMH) • Arcuate nucleus (Arc)
	Lateral	• Lateral nucleus (LT) • Lateral tuberal nuclei
Posterior	Medial	• Mammillary nuclei (part of mammillary bodies) (MB) • Posterior nucleus
	Lateral	• Lateral nucleus (LT)

The primary nuclei within the hypothalamus involved in feeding behaviour (hunger and satiety) include the arcuate nucleus (**Arc**), the dorsomedial hypothalamic nucleus (**DMH**), the ventromedial hypothalamic nucleus (**VMH**) and the paraventricular nucleus (**PVN**; Figure 2.8). Experiments involving lesions in the hypothalamus have demonstrated that the lateral hypothalamic area **(LHA)** is responsible for transmitting orexigenic signals, as loss of this region results in

hypophagia and emaciation (Hetherington and Ransob, 1940). Whereas, the medial hypothalamic nuclei (VMN and to a lesser degree DMN) are responsible for satiety, as loss of this region results in hyperphagia and obesity (Hetherington and Ransob, 1940).

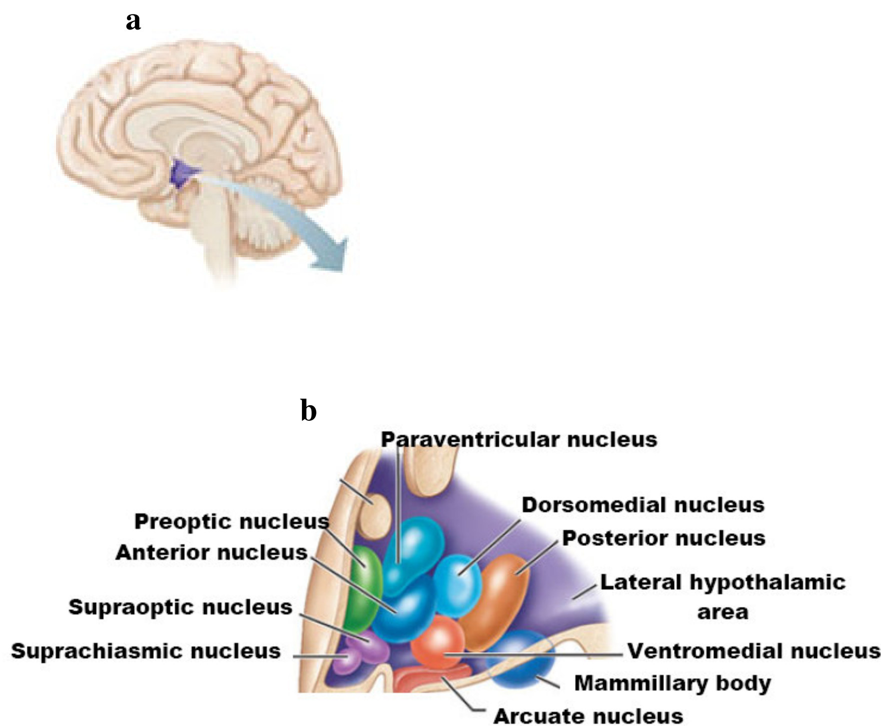


Figure 2.8 Location of hypothalamus within the brain (a), anatomical structure of nuclei within the hypothalamus (b).

The Arc is a collection of neuronal cell bodies situated at the base of the third ventricle and is considered the "master hypothalamic centre' for feeding control (Arora and Anubhuti, 2006). The Arc neurons are called 'first order neurons' due to their direct contact with peripheral hunger and satiety factors. This is due to the MeE, which overlies the Arc, being fenestrated; Arc axon terminals are in direct contact with the blood stream (Peruzzo et al., 2000). The two distinct groups of neurones within the Arc express either the orexigenic (intake promotion) neuropeptide Y (**NPY)** and agouti-related protein (**AgRP**) or the anorexigenic (intake inhibition) proopiomelanocortin

(**POMC**) and cocaine and amphetamine regulated transcript (**CART**). Proopiomelanocortin is the pre-cursor molecule for melanocortins (**MC**), with α-melanocyte-stimulating hormone (**α-MSH**) the predominant MC anorexigenic peptide (Forbes et al., 2001)**.**

Interactions between the Arc neurones allow the NPY neurones to control the activity of the POMC cells (i.e. release of melanocortins) via two mechanisms (Broberger, 2005):

1. NPY neurones co-express AgRP, which is only expressed in the Arc and is a melanocortin antagonist (Yang et al., 1999).
2. The release of melanocortin (i.e. α-MSH) can be blocked at the axon terminal on the POMC neurons with the simultaneous release of AgRP (i.e. AgRP levels ↑ α-MSH ↓ and vice versa).
3. At the cell body level, POMC neurones are innervated by NPY terminals that express the Y1 receptor (Broberger et al., 1997) through which NPY inhibits MC release.

From the Arc, neurons project to 'second-order neurones' in the PVN, VMH, DMH and LHA (Schwartz et al., 2000). Neuropeptides released from the second order neurones include corticotropin-releasing hormone (**CRH**) and thyrotropin-releasing hormone (**TRH**), both of which inhibit feeding behaviour, or melanin-concentrating hormone (**MCH**) and orexin, which stimulate feeding. Second-order neurones project, amongst others, to the NTS and the dorsomotor nucleus of the vagus (**DMV**) nerve. This communication between hypothalamic nuclei and the brainstem, responding to hunger and meal-related satiety signals, is essential in initiating and terminating feeding behaviour.

2.3.3.4 Brainstem - Vagus Nerve

The gut-brain signalling route runs through afferent vagal nerves that transmit signals to the CNS from a variety of sensors in the GI tract, which respond to

mechanical (distension and contraction) stimuli, chemicals, nutrients in the gut lumen, and neuro-hormonal stimuli, such as gut hormones, and neurotransmitters (Konturek et al., 2004). These signals are integrated within the individual vagal sensory neurones and synapse (terminate) onto the NTS, stimulating neurones (Harding and Leek, 1973). Some signals are transmitted onward to the higher neural centres, via the ascending tract from the NTS to the hypothalamus to influence satiation and meal termination (Konturek et al., 2004; Broberger, 2005).

The primary function of the extensive network of vagal afferent terminals located in the smooth muscle throughout the GI tract is the detection of changes in tension (Iggo, 1954, 1955). The tension receptors monitor both the contractions of the muscle wall, through motility and propulsion of digesta through the gut, and the stretch of the wall as determined by the volume of gut contents. Therefore, two different forms of "tension" are encountered by nerve endings: active tension, which occurs when force develops during the contraction of muscle, and passive tension, which develops when a non-contracting muscle is extended (Jami, 1992). The need to distinguish between active and passive tension is critical to GI function (Phillips and Powley, 2000).

2.3.4 The Central Regulation of Food Intake

Energy homeostasis is regulated within the brain, with the first step being to communicate the metabolic state of the individual to the brain; this is achieved through two main channels.

1. Signals reflecting the availability of and demand for metabolic food are relayed via neurones in the hypothalamus, primarily from the neurones expressing NPY (intake stimulating) and POMC (intake inhibition).
2. The afferent vagus nerve relays information from the gastrointestinal tract and other associated organs to the brainstem, whereby, vagal afferents synapse onto (terminate) the NTS, exciting neurones. These signals are projected further into other areas (e.g. PVN, VMH) to initiate or terminate food intake.

Additional to neuropeptides in regulating food intake, other factors within the hypothalamus integrate to either stimulate or inhibit feed behaviour.

2.3.4.1 Neurotransmitters

Within the hypothalamus, the key chemical mode of communication between neurones is via amino acid transmitters (i.e. excitatory glutamate and inhibitory ϒ-amino butyric acid; **GABA**). Their importance is highlighted by studies wherein the absence of glutamate- and GABA–mediated transmission results in very little hypothalamic synaptic activity (Decavel and Van den Pol, 1990; van den Pol et al., 1990). Studies indicate that within the Arc, NPY neurones largely contain GABA, whereas POMC neurones signal via glutamate (Horvath et al., 1997; Collin et al., 2003). Electrical excitation has been proposed to come from loss of inhibition via NPY-mediated suppression of GABA (Cowley et al., 1999; Pronchuk et al., 2002), with MC stimulation producing the opposite result, (i.e. inhibition, via stimulation of GABA release; Cowley et al., 1999).

2.3.4.2 Metabolic Enzymes

Within the Arc, a number of enzymes involved in fatty acid metabolism are expressed, despite the fact that fatty acids are not a major fuel source for this tissue (Lopaschuk et al., 2010). Of these enzymes, malonyl CoA has been implicated as an important contributor to the regulation of food intake (Loftus et al., 2000; Gao and Lane, 2003). Malonyl CoA is generated by the carboxylation of acetyl CoA by acetyl CoA carboxylase (**ACC**) and signals an 'energy surplus' when increased and an 'energy deficit' when its concentration is low (Gao and Lane, 2003). Leptin increases malonyl CoA concentrations within the Arc, by activating ACC, and this up-regulation is thought to mediate the anorexigenic effects of leptin (Gao et al., 2007). Additionally, increases in hypothalamic malonyl CoA are linked to down-regulation of NPY/AgRP expression and up-regulation of POMC/CART and vice versa (Loftus et al., 2000; Gao and Lane, 2003; Gao et al., 2007). During states of fasting, hypothalamic levels of malonyl CoA rapidly decrease and act as a signal of hunger, whereas during feeding,

levels increase and act as a signal to stop eating (Wolfgang and Lane, 2006). The level of hypothalamic malonyl CoA plays an important role in the activation of adenosine 5'-monophosphate-activated protein kinase (**AMPK**), which is proposed to be the "master switch" in the regulation of food intake, as it monitors cellular energy status (Minokoshi et al., 2004). The activation of AMPK within the Arc promotes food intake, whereas inhibition has the opposite effect. Neuropeptides themselves can increase or decrease activation of AMPK within the hypothalamus. Increasing levels of leptin and insulin result in the inhibition of AMPK, resulting in increased ACC activation and increased malonyl CoA concentrations (i.e. inhibit intake). In comparison, NPY may increase AMPK, resulting in a reduction in malonyl CoA; however, studies do not conclusively support this. Ghrelin (Anderson et al., 2008) and AgRP (Minokoshi et al., 2004) activate AMPK, inhibiting ACC activity and subsequent malonyl CoA production (i.e. stimulate intake). The critical role for AMPK in intake regulation is highlighted by its necessary inhibition for leptin's anorexigenic effects to effect feeding behaviour (i.e. increased concentrations of AMPK block leptin's effects; Minokoshi et al., 2004).

2.3.5 Integration of peripheral signals

The GI tract is the body's largest endocrine organ, releasing more than 30 recognised regulatory peptide hormones that influence a number of physiological processes and act on tissues, including exocrine glands, smooth muscle and the peripheral nervous system (Murphy et al., 2006). Although their role in the regulation of gastrointestinal function has been known for decades, there is increasing evidence that some may also influence eating behaviour (Murphy et al., 2006).

Peripheral signals act upon the Arc and NTS to influence the central pathways regulating short-term intake regulation, on a meal-to-meal basis. This is facilitated by the release of several key gut hormones to initiate or terminate a meal. Several key gut hormones (Table 2.3) such as ghrelin, cholecystokinin (**CCK**), peptide tyrosine tyrosine (**PYY**), glucagon-like peptide-1 (**GLP-1**) and oxyntomodulin (**OXM**), are released from the intestinal endocrine cells and act on their respective receptors on the gastric vagal afferent nerve and directly on Arc neurons. Additionally, adipose tissue and the

pancreas release leptin and insulin, respectively, in response to food intake. The fluctuation of these hormones/peptides has been proposed to mediate feeding behaviour, depending on whether the individual is in a fasted, pre-prandial or a fed-state.

For a more information on key hormones and peptides involved in intake regulation, including size, function and infusion studies, please refer to Appendix A1 and A2.

Table 2.3 Summary of key gastrointestinal neuropeptides.

Hormone	Location	Major Effect
Cholecystokinin (CCK)	Enteroendocrine I cells in the duodenum and jejunum	Increase gastric acid secretions, increase intestinal motility, and inhibit gastric emptying
Ghrelin	X/A-like enteroendocrine cells of the oxyntic glands, minor release in intestine, pancreas and hypothalamus	Stimulates feed intake, increases gastric emptying,
Peptide tyrosine tyrosine (PYY)	Enteroendocrine L cells in the ileum and colon	Reduces gut motility, delays gastric emptying inhibits gastric and pancreatic secretion, induces satiety
Glucose-dependent insulinotropic polypeptide (GIP)	Enteroendocrine K cells of the duodenum and jejunum	Inhibits gastric acid secretion, reduce gastric motility, enhances post-prandial insulin secretion
Glucagon-like peptide- 1 (GLP-1)	Enteroendocrine L cells in the ileum and colon	Potentiates glucose-dependent insulin secretion, inhibits glucagon secretion, inhibits gastric emptying
Oxyntomodulin	Enteroendocrine L cells in the ilium and colon	Augment post-prandial insulin secretion, inhibit gastric acid secretion, reduce gastric motility
Pancreatic polypeptide (PP)	F cells within pancreatic islets cells	Delay gastric emptying

Table 2.4 Summary of key neuropeptides involved in intake and their effects on feed intake in a normal, intracerebroventricular administered and gene knockout scenarios.

(Abbreviations on following page)

Neuropeptide	Expression Site	Central Receptor	Physiological Effect on Intake	ICV Effect	IV Administration	Knockout Effect
First Order Neurons Neuropeptides						
POMC	Arc, NTS					↑
α-Melanocyte-stimulating hormone (α-MSH)	Arc	MS3-R, MS4-R	↓	↓		
Cocaine & amphetamine regulated transcript (CART)	Arc,PVN, DMH, LHA, ME		↓	↓		NE
Neuropeptide Y (NPY)	Arc	Y1-5	↑	↑		↓
Agouti-related peptide (AgRP)	Arc	MC4-R*, MCR-3*	↑	↑		
Second Order Neurons Neuropeptides						
Melanin concentrating hormone (MCH)	LHA	MCH-R		↑		
Orexin A	LHA	Ox1-R, Ox2-R		↑		↓
Orexin B	LHA	Ox1-R, Ox2-R		↑/NE		↓
Corticotropin releasing hormone (CRH)	PVN	CRH1-2		↓		
Peripheral Hormones/Peptides						
Ghrelin	Stomach, Arc	GHS-R	↑	↑	↑	
Insulin	Pancreas	Ins-Rb	↓	↓		↑
Leptin	Adipose	Ob-Rb	↓	↓		↑
Cholecystokinin (CCK)	S. Int	CCKA-R, CCKB-R	↓	↓/NE		
Peptide tyrosine-tyrosine (PYY)	S. Int.	Y2	↓	↓	↓	
Pancreatic Polypeptide (PP)	Pancreas	Y4	↓	↓		
Glucagon-like peptide (GLP-1)	S. Int, NTS,	GLP1-R	↓	↓	↓	

↓↑	Arrows indicate increase or decrease in food intake
ICV	Intracerebroventricular administration
Arc	Arcuate nucleus
NTS	Nucleus Solitary Tract
PVN	paraventricular nucleus
LHA	lateral hypothalamic area
DMH	Dorsomedial nucleus
GHS-R	G-protein coupled receptor
MC3-4 -R	Melanocortin 3-4 receptor
Ob-R_b	Leptin receptor
Ins-R_b	Insulin receptor
Y1-5	NPY and PYY receptor
MCH-R1-2	Melanin concentrating hormone receptor
Ox-1-2	Orexin receptors
*	Potent antagonist
NE	No effect
S. Int	Small Intestine

2.3.5.1 In a Fasted State

During a fasted or pre-prandial state, AgRP and NPY gene expression increases and POMC gene expression decreases within the hypothalamus (Li et al., 2000). The X/A cells within the oxyntic mucosa (in the stomach) express and release the hunger-stimulating orexigenic peptide, ghrelin (Tschop et al., 2000). Peripheral ghrelin crosses the BBB through the MeE (Kojima and Kangawa, 2005), activating its respective G-protein coupled receptor (**GHS-R**), which is co-localised with NPY in the Arc (Kojima et al., 1999), to promote the expression and release of hypothalamic NPY and AgRP (Greenman et al., 2004). The increased concentration of NPY stimulates the LHA to release orexin or MCH, via the NPY1-receptor pathway, thereby enhancing feed intake (Broberger et al., 1997). The low circulating leptin and insulin concentrations stimulates increased concentrations of AgRP within the Arc, which bind to MC3-R and MC4-R, the receptors for MC, acting as a potent antagonist (Hillebrand et al., 2002b), inhibiting the release of MC, in particular α-MSH. Low circulating glucose increases activity to the hepatic vagal afferent (Niijima, 1969). Whereas, the gastric afferent vagal nerves are inhibited due to:

- Increased ghrelin concentration (inhibitory factor; Date et al., 2002).
- Low circulating concentrations of satiety factors such as CCK, PYY, OXM, and GLP-1 (stimulatory factors).
- Decreased stimulation of the mechanoreceptors in the stomach and small intestine (Iggo, 1954, 1955)

Signals (activity, both inhibitory and stimulatory) to the NTS are projected to the hypothalamus, to stimulate the release of orexin or MCH, thus stimulating feed initiation, and within the visceral sensory complex (**VSC**), of the thalamus, evoking a perception of hunger (Ahima and Antwi, 2008). These combined signals from the periphery via the afferent vagal nerves and within the hypothalamus and thalamus alert the individual to ingest food (Figure 2.9).

Figure 2.9 Diagram illustrating hypothalamic and peripheral activity in a fasted or pre-prandial state. In a fasted or pre-prandial state, the stomach releases increased concentrations (conc) of ghrelin that enter circulation and cross the blood brain barrier via the median eminence and binds to its receptor on the NPY/AgRP neurone, within the arcuate nucleus (Arc) in the hypothalamus. This stimulates the expression and release of NPY and AgRP. Increased NPY concentration stimulates second order neurones in the LHA and PVN to release the orexigenic neuropeptides orexin and MCH, whilst inhibiting release of the anorexigenic neuropeptides CRH and TRH from the PVN, respectively. Additionally, the low circulating concentration of insulin and leptin also stimulate the release of AgRP that binds to the MC receptor on the POMC neurone, inhibiting the release of α-MSH. The simultaneous stimulatory and inhibitory activity from peripheral sites to the NTS, via the vagus nerve, project to the LHA stimulating release of MCH and orexin, and to the VSC in the thalamus that brings about the perception of hunger. These combined hypothalamic and peripheral signals induce feed intake.

In a fasted or pre-prandial state

Feed Intake Stimulated

Thalamus

VSC

Perception of Hunger

Hypothalamus

PVN

CRH

TRH

Orexin

MCH

LHA

Arc

αMSH

POMC

NPY

AgRP

NPY/AgRP

Median eminence

NTS

Circulation

Vagus Nerve

↓ Leptin

↓ Glucose

↑ Ghrelin

↓ Distension
↓ Contraction

↓ Insulin

↓ PYY
↓ CCK
↓ OXM

Adipose Tissue

Liver

Stomach

Intestine

Pancreas

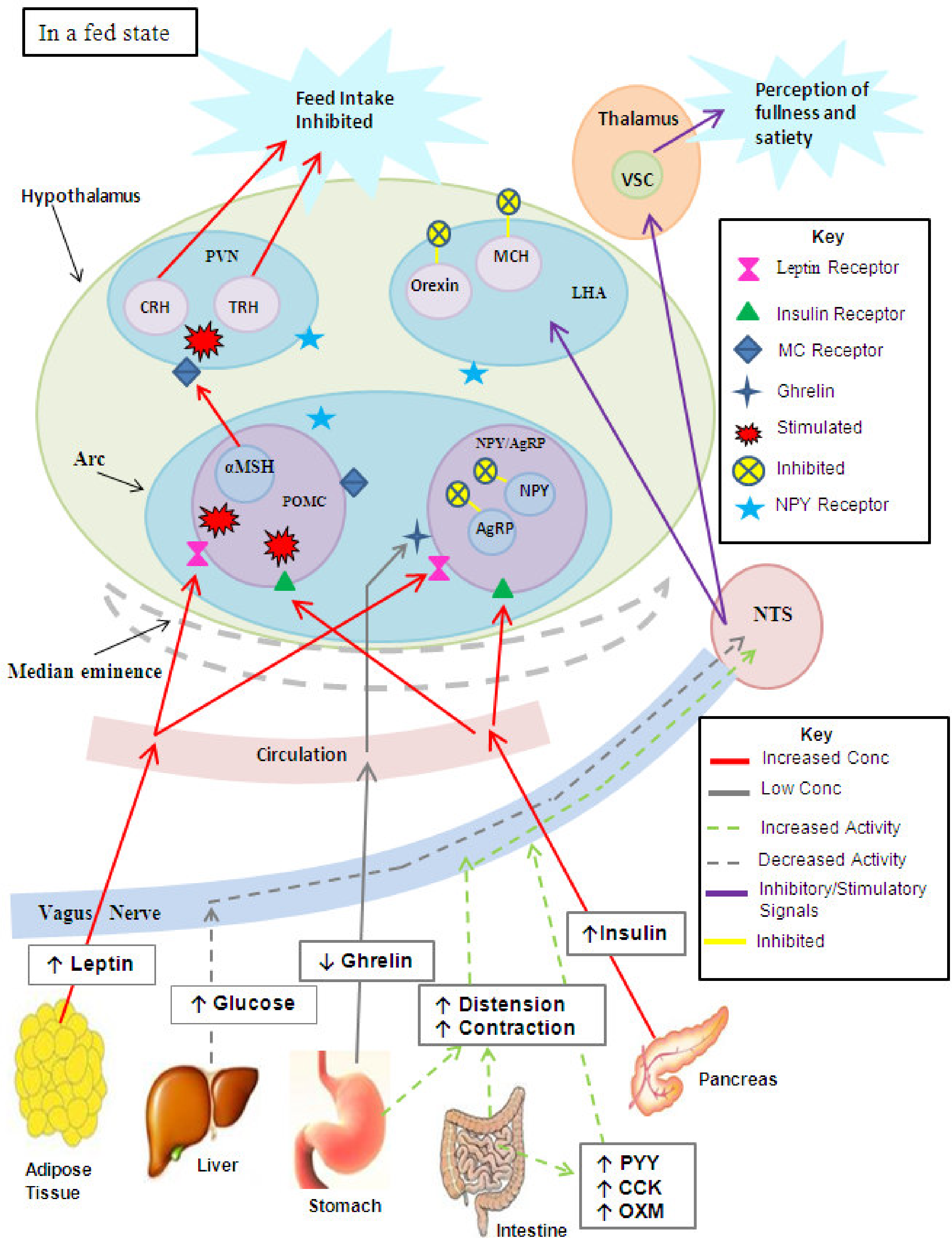
Key
Leptin Receptor
Insulin Receptor
MC Receptor
Ghrelin Receptor
Stimulated
Inhibited
NPY Receptor

Key
Increased Conc
Low Conc
Increased Activity
Decreased Activity
Inhibitory/Stimulatory Signals
Inhibited

2.3.5.2 In a Fed State

The consumption of food initiates a sequence of neuronal and hormonal responses within the GI tract and associated organs. Even before the ingestion of food, the very sight or smell of an impending meal stimulates exocrine and endocrine release from the gut, as well as an increase in gut motility in preparedness for incoming nutrients; this is known as the cephalic phase response (Pavlov, 1902; Powley and Berthoud, 1985). Upon the ingestion of food, mechanoreceptors in the stomach are stimulated, because of distension to accommodate intake, and increase the stimulatory activity of the gastric afferent vagus nerve (Iggo, 1954, 1955). The increased glucose concentration in circulation with carbohydrate digestion decreases the activity of the hepatic afferent vagal nerves (Niijima, 1969). As the digesta moves through the GI tract, peripheral signals enter circulation from regions of the small intestine (e.g. CCK, and PYY), pancreas (insulin and PP), and adipose tissue (leptin) that stimulate gastric vagus afferents and the Arc neurones directly. Increasing levels of leptin and insulin signal the brain that excess energy is being stored, and this brings about adaptations of decreased hunger (satiety). The binding of leptin and insulin to their respective receptors (Ob-R and Ins-R) on the NPY and POMC neurones in the Arc inhibit transcription and release of NPY/AgRP (Schwartz et al., 1991; Ahima et al., 1996). This increases POMC activity and α-MSH expression, inducing satiety through inhibition of NPY pathway in the PVN (Schwartz et al., 1997; Thornton et al., 1997; Kim et al., 1999). As well as acting on the Arc, peripheral signals (hormonal and mechanoreceptor) stimulate an increased discharge on the gastric afferent vagal nerves (Iggo, 1954, 1955; Date et al., 2002) terminating on the NTS, stimulating neurones. Projections from the NTS enter the hypothalamus to inhibit the PVN and release CRH and TRH, inhibiting feed intake, and the VSC of the thalamus, which brings about the perception of GI fullness and satiety (Ahima and Antwi, 2008). These combined signals alert the individual to terminate feeding (Figure 2.10).

Figure 2.10 Diagram illustrating hypothalamic and peripheral activity in a fed state. After the consumption of food, the concentrations of leptin and insulin increase, while ghrelin concentration decreases. The increased concentrations of leptin and insulin stimulate POMC neurones, and inhibit the NPY/AgRP neurone promoting release of the anorexigenic neuropeptide α-MSH (derived from POMC) that binds to its receptor in the PVN stimulating release of anorexigenic neuropeptides CRH and TRH, inhibiting neuropeptide release from the LHA. The simultaneous stimulatory and inhibitory activity from peripheral sites to the NTS, via the vagus nerve, project to the LHA inhibit release of MCH and orexin, and to the VSC in the thalamus that brings about the sensation of fullness and satiety. These combined hypothalamic and peripheral signals terminate feed intake.



In a fed state
Feed Intake Inhibited
Thalamus
VSC
Perception of fullness and satiety
Hypothalamus
PVN
CRH
TRH
Orexin
MCH
LHA
Arc
αMSH
POMC
NPY/AgRP
NPY
AgRP
Key
Leptin Receptor
Insulin Receptor
MC Receptor
Ghrelin
Stimulated
Inhibited
NPY Receptor
NTS
Median eminence
Circulation
Key
Increased Conc
Low Conc
Increased Activity
Decreased Activity
Inhibitory/Stimulatory Signals
Inhibited
Vagus Nerve
↑ Leptin
↑ Glucose
↓ Ghrelin
↑ Insulin
↑ Distension
↑ Contraction
Pancreas
Adipose Tissue
Liver
Stomach
Intestine
↑ PYY
↑ CCK
↑ OXM

2.3.6 Intake Regulation in Ruminant Species

Due to anatomical GI differences between ruminant and monogastric species and the constant influx of digesta entering the gut, the function and secretion of gut-derived peptides may differ in the ruminant animal compared with the discrete meals of monogastric species. Infusion studies both centrally and peripherally in monogastric species identified intake regulatory roles for a number of hormones and peptide (i.e. leptin, NPY, ghrelin etc.); however, their role in ruminant species, particularly under grazing conditions remains less clear.

Intracerebroventricular (**ICV**) infusion of NPY increased DMI in feed-satiated sheep, with a response within 30 min of administration and lasting for 2-3 h (Miner et al., 1989). The peripheral plasma concentration of NPY increased when splanchnic nerves were stimulated in calves (Allen et al., 1984), and long term feed restrictions in sheep increasing NPY expression in the Arc and PVN, (Barker-Gibb and Clarke, 1996; Polkowska and Gładysz, 2001). Dry matter intake also increased with ICV and peripheral administration of ghrelin (Harrison et al., 2003; Wertz-Lutz et al., 2006), with the orexigenic actions of ghrelin mediated via NPY neurons, as similarly reported in monogastric species. Additionally, similar intake regulatory roles have been reported for insulin and leptin in the ruminant and monogastric species; DMI was reduced when insulin was peripheral or centrally administered; (Deetz and Wangsness, 1981; Foster et al., 1991) and when leptin was centrally administered in sheep (Morrison et al., 2001). However, circulating concentrations of PYY did not fluctuate in sheep over a 48 h period, despite PYY cell distribution in the mucosa of the lower intestinal tract (Onaga et al., 2000), and peripheral administration of CCK did not reduce food intake (Grovum, 1981) in sheep. In comparison, peripheral administration of CCK in humans increased the sensation of fullness and decreased food intake in a dose-dependent manner (Kissileff et al., 1981; Muurahainen et al., 1988; Lieverse et al., 1994). Interestingly, it is the hormones released from the GI tract implicated in satiety in monogastric species, which do not appear to have the same effects in ruminant species; this may stem from the continuous flow of digesta through the GI tract.

Satiety in ruminant species is proposed to be a result of hepatic oxidation of a variety of fuels (Forbes, 1988), stimulating the hepatic vagus nerve inducing satiety; this is known as the hepatic oxidation theory (**HOT**; Allen et al. 2009). Hepatic oxidation increases throughout a meal, increasing the energy status of hepatocytes and decreasing the discharge rate of the hepatic vagal afferents, thereby inducing satiety. Hepatic oxidation declines after meals, decreasing the energy status of the hepatocyte, increasing the discharge rate of hepatic vagal afferents and facilitating the sensation of hunger (Forbes, 1992). Signals that relate to the energy status of hepatocytes to the hepatic vagal afferents are integrated in the NTS before being communicated to the hypothalamus (Forbes, 1992).

Niijima (1969) reported that the firing rate of the hepatic afferent vagal nerve was inversely related to the concentration of glucose in the blood. However, due to the low hepatic uptake of glucose from circulating blood in adult ruminants (Stangassinger and Giesecke, 1986) other fuel sources are oxidized by liver tissue, decreasing the hepatic afferent vagal nerve firing rate, and, in theory, inducing satiety. Propionate, a major VFA, is the primary glucose precursor in ruminants, and can account for up to 80% of glucose production in the lactating cow (Steinhour and Bauman, 1988). Along with being converted to glucose, propionate can be oxidized in the TCA cycle (Steinhour and Bauman, 1988), as well as stimulate oxidation of acetyl CoA from other fuels sources (Allen, 2000). The ruminant liver has a high activity of propionyl CoA synthetase, which is necessary for the metabolism of propionate, and, therefore, propionate is extensively metabolised in the liver (Armentano, 1992).

Studies investigating the effects of VFA on hepatic oxidation and satiety (hypophagia) have reported that infused propionate, but not acetate or butyrate (Knapp et al., 1992; Oba and Allen, 2003), reduced food intake. Oba and Allen (2003) ruminally infused propionate and reported a linear reduction in meal size 2.5 to 1.5 kg DM as propionate increased. Elliot et al. (1985) infused propionate and acetate separately into the mesenteric vein, and reported a reduction in DMI when propionate was infused, but no effect with acetate. Dietary FA can reduce DM and energy intakes, but a role for hepatic oxidation in the hypophagic effects has not been demonstrated in ruminants (Allen, 2000). The most indirect evidence for hepatic oxidation of FA is the

reduced DMI for ruminants in a lipolytic state, due to the elevated non esterified fatty acids (**NEFA**) concentrations, and subsequent NEFA oxidation in the liver (Allen et al., 2009). Although numerous studies have been undertaken in ruminant species, intake regulation remains unclear.

The literature reviewed has highlighted a substantial body of information on intake regulation; however, the vast majority of this is in monogastric species. Dry matter intake regulation remains less clear for ruminant species, and especially in grazing ruminants. Special differences are most likely due to anatomical differences in the GI tract and the constant flow of digesta in ruminant species compared with monogastric species. The role of humoral factors on feeding behaviour of grazing ruminants needs to be established, as does the effect of supplementation on these parameters. Understanding these factors may help increase DMI, milk production and response to supplements in grazing systems.

Therefore, the objectives of the proposed research are to:

1. Understand variations in grazing behaviour in pasture-fed cows and the effects that supplementation have on grazing time throughout the day during early, mid and late lactation.
2. To determine whether the time that grazing dairy cows are supplemented (either at a.m. or p.m. milking) alters daily grazing behaviour, pasture DMI and milk production.
3. Profile diurnal humoral profiles known to be associated with intake regulation in monogastric species and investigate associations in pasture-fed dairy cows.
4. To determine if changes in feeding behaviour in pasture-fed dairy cows coincide with changes in humoral factors using an intensive blood-sampling regime that coincide with the major feeding bouts post-sunrise and pre-sunset. In addition, to investigate the effects of supplement type on feeding behaviour, pasture DMI and the profile of humoral factors.

Chapter 3
Genetic Strain and Diet Effects on Grazing Behaviour, Pasture Intake and Milk Production.

.

3.1 Abstract

Understanding how dairy cows adjust their grazing behaviour in response to feed supplements is important for the development of management strategies that optimise profit from supplementation. New Zealand (**NZ**) HF cows have been selected for milk production on a predominantly pasture-based diet; in comparison, HF cows of North American (**NA**) ancestry have been selected almost exclusively for milk yield and fed diets high in non-fibre carbohydrates (**NFC**). It was, therefore, hypothesized that supplementation would have differing effects on grazing behaviour, pasture DMI, and milk production in these genetic strains at peak, mid, and late lactation. A study was conducted over two consecutive lactations with NA and NZ cows randomly allocated at calving to 0, 3, or 6 kg DM/day concentrate plus unrestricted access to pasture. Pasture DMI, milk production and grazing behaviour were recorded at peak, mid, and late lactation. Concentrates were fed in equal amounts at a.m. and p.m. milking. The NA cows produced more milk and milk components, and had a greater pasture DMI, despite spending less time grazing. There was a decline in time spent grazing and pasture DMI associated with increasing concentrate DMI. Grazing behaviour following a.m. supplementation was different to that recorded following p.m. supplementation. Grazing ceased following a.m. supplementation before rumen fill could be a limiting factor and the length of the grazing interval was inversely proportional to the amount of concentrate offered; these results suggest physiological rather than physical stimuli were responsible for grazing cessation. The decrease in time spent grazing with increasing concentrate DMI is consistent with changes in neuroendocrine factors secreted in response to the presence of food in the digestive tract or with circulating products of digestion. After p.m. supplementation, sunset signalled the end of grazing irrespective

of stage of lactation, timing of sunset, or supplementation status, suggesting photoperiod influenced grazing behaviour. Results confirmed changes in grazing behaviour, an associated reduction in pasture DMI, and an increase in milk production when cows consume increasing amounts of concentrates. However, as the effect of supplement on grazing behaviour differed between a.m. and p.m. supplementation, further research is required to understand better the factors controlling grazing behaviour, to allow improved milk production responses to supplementary feeding.

3.2 Introduction

Low DMI is a major limitation to productivity in pasture-based dairy systems resulting in nutrient intakes that are insufficient to exploit the genetic capability of the lactating cow for milk production (Kolver and Muller, 1998). Understanding how cows adjust their grazing behaviour to contend with the changing environment and forage dynamics is important for the development of management strategies that optimise dairy cow production (Demment et al., 1986). For example, the objective of feeding supplements to grazing cows is to increase total DM and ME intakes, compared with those achieved on pasture alone (Stockdale, 2000b). However, feeding supplements can have a marked effect on pasture DMI, with pasture DMI reported to decline with increasing supplementation (Bargo et al., 2003).

The decline in pasture consumed relative to the amount of supplement eaten is referred to as substitution and can result in poor marginal responses to the supplement provided. Bargo et al. (2003) reported that, on average, feeding concentrates reduced grazing time by 12 min/kg DM concentrate offered. This is a major factor contributing to the variable milk yield response to concentrate supplementation (marginal milk response; Stockdale, 2000b). Substitution rate (**SR**) has been reported to decline from spring to summer to autumn (Stockdale, 2000b), suggesting the negative effect of supplementation on grazing behaviour may vary with stage of lactation, or with seasonal variations in pasture quality.

Genetic differences may also contribute to the SR and milk response (**MR**), as NZ HF cows are fed a predominantly pasture-based diet with very limited concentrate

supplement, are lighter, and produce less milk volume, but have better fertility and survival (Harris and Kolver, 2001). Whereas, NA HF cows are larger and produce more milk with lower concentrations of fat and protein, and have poorer fertility and survival than NZ HF cows (Roche et al., 2006a). Holstein Friesian cows of NA ancestry were, until recently, selected almost exclusively for milk yield and fed diets high in NFC (Harris and Kolver, 2001). Previous studies comparing these strains have also reported a lower pasture DMI in NZ cows and a lower milk response (**MR**) to supplements than their NA comparison (Horan et al., 2004; Linnane et al., 2004). The two HF strains, therefore, provide a comparison of the effects of genetic selection on cow behaviour.

Pasture DMI depends on factors that govern commencement and cessation of successive grazing bouts (Gregorini et al., 2006). Grazing is predominately a daylight activity, with 65-100% of daily grazing time reportedly between 0600 and 1900 h over a wide range of environmental temperatures, supplementation regimes, grazing managements, and pasture DMI (Krysl and Hess, 1993). However, this does not mean that grazing behaviour is unresponsive to environmental and management cues. For example, in the a.m., dairy cows interrupt their first grazing bout long before reaching maximal rumen capacity (Taweel et al., 2004) and bite rate is reported to be greatest during the evening meal, so that cows maximize DMI before darkness (Gibb et al., 1998). Although the effect of concentrates on average grazing time and bite rate has been reported (O'Connell et al., 2000; Bargo et al., 2003), little is known about differences in grazing and ruminating behaviour in response to cow genetics, supplementation at different times of the day (Krysl and Hess, 1993), and stage of lactation.

This experiment investigated the effect of feeding concentrates on grazing behaviour, pasture DMI, and milk production of NZ and NA HF cows at peak, mid and late lactation over two years. It was hypothesised that the a.m. allocation of concentrates would decrease grazing time to a greater extent than the p.m. allocation, due to the findings that bite rate increases at the evening meal. To test this, grazing and ruminating activity following a.m. and p.m. allocation of concentrates were investigated separately.

3.3 Materials and Methods

This experiment was conducted at Lye Farm, DairyNZ, Hamilton, New Zealand from July 2002 to June 2004 and the Ruakura Animal Ethics Committee, Hamilton, New Zealand, approved all procedures.

3.3.1 Experimental Design

The experimental design was reported in detail by (Roche et al., 2006a). Briefly, over two years (**yr**), 54 and 59 (yr 1 and 2, respectively) primiparous and multiparous HF cows of NA (n=27 and 29 in yr 1 and 2, respectively) and NZ (n= 27 and 30 cows in yr 1 and 2, respectively) ancestry were randomly allocated, at calving, to one of three supplementary feeding treatments in a 2 X 3 factorial arrangement. Cow allocation ensured that all treatments were balanced for age (5.1 ± 1.60 yr and 5.4 ± 1.68 yr in yr 1 and 2, respectively), calving date (July 28 ± 19.9 day (**d**) and July 27 ± 26.0 d in yr 1 and 2, respectively), and breeding worth (measure of genetic merit accounting for the economic value of the trait (Harris et al., 1996). Cows were re-randomized at the beginning of yr 2, ensuring that treatment groups were again balanced for the same criteria as yr 1.

All cows were offered a > 45 kg DM/cow per day of fresh pasture (to ground level) and 4 of the 6 treatments (2 from each genetic strain) received either 3 or 6 kg DM of a pelleted grain-based concentrate/day. The remaining two treatments received no concentrate. Treatments were therefore, NZ0, NZ3, NZ6, NA0, NA3, and NA6.

3.3.2 Genetic strains

The two genetic strains were described in detail by Roche et al. (2006a). Briefly, the NA strain cows had >87.5% NA genetic ancestry and were either imported from the United States or the Netherlands as embryos by Holland Genetics Ltd. for LIC, New Zealand or were direct descendants of the imported embryos. The mean estimated breeding values (**EBV**) for the NA cows on study were +1,270 (standard deviation; **sd** 246.4) kg milk, +32 (sd. 7.7) kg fat, +39 (sd 6.4) kg protein, +93 (sd 12.4) kg body weight (**BW**) and -40 (sd 117.1) day survival. The NZ cows used in the present

experiment were selected from DairyNZ herds based on their breeding worth and the proportion of NZ ancestry (<12.5% NA genes). The EBV for the NZ cows on study were +820 (sd. 225.5) kg milk, +29 (s.d 6.95) kg fat, +28 (sd 5.90) kg protein, +52 (sd 15.8) kg BW and +325 (sd 79.92) day survival. Each strain represented 6 to 9 sires, which were common across feeding treatments within strain.

3.3.3 Pasture Management and Supplementary Feeding Treatments

Cows were rotationally grazed as one herd for the duration of the experiment and only returned to the same area when a minimum of 2 leaves had appeared on the majority (>75%) of perennial ryegrass tillers. Cows had access to a fresh allocation of pasture after each milking. Pasture allowance (> 45 kg DM/cow per day) was sufficient to ensure unrestricted DMI (up to approximately 25 kg DM/day) of fresh pasture in the unsupplemented cows. Pasture was of high quality throughout both years (crude protein **CP** = 22.2 ± 2.73% DM; organic matter (**OM**) digestibility = 84.2 ± 3.87% DM; NDF = 40.0 ± 4.38% DM; acid detergent fibre (**ADF**) = 22.2 ± 2.50% DM; lipid = 4.1 ± 0.25% DM; non-structural carbohydrates (**NSC**) = 11.3 ± 2.50% DM; ME = 11.8 ± 0.54 mega joules **MJ**/kg of DM). Pasture quality was maintained throughout the season, despite the high grazing residuals, through strategic mowing.

A flat rate of either 3 or 6 kg DM of concentrates (60% crushed corn; 32% crushed barley; 6 % molasses; 2% wheat middlings; CP =11.2 ± 1.46% DM; NDF = 9.8 ± 1.99% DM; lipid = 2.7 ± 1.22% DM; NSC = 71.9 ± 2.16% DM) was fed individually to the appropriate treatments. Concentrate allocation was split equally in two feeds and offered daily during milking. For 15 d pre-calving, all cows were offered 2 kg DM/d concentrate. Following calving, the NZ3 and NA3 cows received 3 kg DM/day concentrate and the NZ6 and NA6 were gradually stepped up to 6 kg DM/day concentrate over the following 6 d (0.5 kg DM/day concentrate).

3.3.4 Milk Production

Milk production results for this study are for the periods that pasture DMI and grazing behaviour were analysed. Individual milk yields were recorded daily (Westfalia

Surge, Oelde, Germany). Milk fat, CP and lactose concentrations were determined by Milkoscan (Foss Electric, Denmark) on a composite p.m. and a.m. sample collected on one day each week. Milking times were 0630 h and between 1400 – 1430 h, depending on the stage of lactation

3.3.5 Grazing Behaviour

Time spent grazing, ruminating, lying and standing was determined by recording each cow's activity at 10 min intervals throughout a 24 h period while the cows were in the paddock (Gary et al., 1970). Grazing was defined as 'cows in the act of eating'. Behaviour was recorded on two 24 h periods during peak (October), mid (January) and late (April) lactation in each year. This provided 4 x 24 h grazing periods at each stage of lactation across two years. The 24 h observation periods were further divided into four key periods to determine the effect of treatment on cow grazing behaviour at these times. The four periods were: Period one (post a.m. milking to p.m. milking), Period 2 (post p.m. milking to sunset), Period 3 (post sunset to 23:0 h), and Period 4 (0000 h to a.m. milking). The duration of the primary grazing bout following a.m. and p.m. milking was calculated as the difference between the time each cow entered the pasture and the time immediately prior to the recording of two consecutive non-grazing behaviours.

3.3.6 Pasture Intake Measurements

Individual cow DMI estimates were obtained at pasture using the n-alkane technique outlined by (Roche et al., 2008b). Briefly, each cow was dosed twice daily (at milking) for a 10 d period with a pellet containing 356 mg of n-dotriacontane (C32; i.e. 712 mg C32/cow per d). Faecal grab samples were collected twice daily from each cow (after milking) during the last 5 d of the 10 d period. The ten faecal samples from each cow for the 5 d period were then bulked, freeze dried, and stored at $-17^{O}C$ awaiting alkane analysis. During the same 5 d period, pasture samples were "plucked" to grazing height on two occasions each d, following close observation of the grazing animal, to represent pasture grazed. A sample of the concentrate consumed was sampled at the a.m. and p.m. feeding event each day of the 5 d sampling period. The n-

alkane content (C25-C36) of the pasture, supplement and faeces were determined using gas chromatography.

The ratio of pasture C33 (tritriacontane) to dosed C32 (n-dotriacontane) was used to estimate pasture DMI.

$$\text{Daily pasture DMI (kg/cow)} = \frac{F_i/F_j.(D_j + I_S.S_j) - I_S.S_i}{P_i - (P_j.F_i/F_j)}$$

where F_i, S_i, and P_i are the concentrations (mg/kg of DM) of the natural odd-chain n-alkane (C33) in faeces, supplement, and pasture, respectively, F_j, S_j, and P_j are the concentrations (mg/kg of DM) of the dosed even-chain n-alkane (C32) in faeces, supplements, and pasture, respectively, and D_j and I_S are the dose rate (mg/d) of the even-chain n-alkane (C32) and supplement DMI, respectively.

3.3.7 Statistical Analysis

Intake, production and behaviour summary measures were calculated as follows. Each variable was analysed separately using mixed models, including age group (heifer vs. cow), season (yr 1 and 2), genetic strain, diet and the interaction between genetic strain and diet as fixed effects, and cow as a random effect. Mixed models were fitted using residual maximum likelihood (**REML**) and GenStat 12.1 (VSN International, Hemel Hempstead) was used for all statistical analyses. Summary grazing behaviour (Table 3.2) as analysed above was then converted into min spent grazing. Bite mass was estimated by average pasture DMI/time spent grazing. Substitution rate was calculated by regressing pasture DMI (independent variable) against supplement DMI (dependent variable) with the slope equalling SR. There were no significant interaction between genetic strain x diet; therefore, only strain and diet effects are reported.

3.4 Results

The effects of treatment on total and pasture DMI and milk production are presented in Table 3.1. North American cows consumed more ($P < 0.001$) pasture than NZ cows in mid and late lactation, but not in early-lactation. Data indicate that NA

cows reached maximum DMI later than NZ cows. Irrespective of stage of lactation, concentrate supplementation resulted in a linear increase ($P < 0.001$) in total DMI, but a decline ($P < 0.05$) in pasture DMI. There were differences in substitution rates (kg pasture substituted/kg conc. consumed) between strains. The NZ cows had a SR of 0.45 (sd 0.96), 0.72 (sd 0.02), and 0.48 (sd 0.008) at early, mid and late lactation, respectively. Whereas, the NA cows had a SR of 0.29 (sd 0.163), 0.26 (sd 0.178), and 0.17 (sd 0.014) at the same lactation stage, respectively.

North American cows were heavier ($P < 0.001$) at calving, at nadir, and at the end of lactation (data not presented: see Roche et al., 2006a). However, NA cows lost 26% more ($P < 0.001$) BW between calving and nadir than NZ cows (84 vs. 62 kg of BW). Although both strains calved at a similar body condition score (**BCS**), NA cows had a lower ($P < 0.001$) BCS at nadir and dry off, and lost BCS for 14 d longer ($P < 0.01$) than NZ cows; they also gained less BCS post nadir ($P < 0.001$). Concentrate supplementation did not affect nadir BW, but there was a linear increase ($P < 0.05$) in nadir BCS with increasing concentrate supplementation (for more detail, see Roche et al., 2006a).

Consistent with the greater pasture DMI, NA cows produced more milk ($P < 0.001$), but with lower fat and protein percent. Consistent with the higher milk yield, NA cows had higher fat, protein, and lactose yields at all stages of lactation. Concentrate supplementation increased milk yield, but the MR was greater in the NA cows (1.9 and 1.1 kg milk/kg concentrate DM in NA cows compared with 0.8 and 0.7 kg milk/kg concentrate DM in NZ cows in the 3 and 6 kg DM/day groups, respectively). Irrespective of strain, MR (1.3 and 0.9 kg milk/kg concentrate DM at 3 and 6 kg DM/d, respectively) to concentrates was 31% greater on average in cows consuming 3 kg DM/d compared with 6 kg DM/day.

Diurnal grazing and rumination patterns are presented in Figure 3.1 and Figure 3.2, respectively, with summary data presented in Table 3.2. Lying and standing behaviour, although recorded, is not presented. On average, NZ cows spent 20 min/day more grazing and 9 min/day less ruminating than NA cows. An increase in rumination time was recorded with increased level of concentrate. Differences were recorded

during the early hours of darkness with NZ cows spending more time grazing than NA cows (Period 3; Table 3.2). Although NA cows spent less time grazing, their bite mass was, on average, 41 g/min compared with 34 g/min in NZ cows ($P < 0.01$).

Time spent grazing declined linearly ($P < 0.001$) with increasing concentrate DMI. Cows supplemented with 3 kg DM/day spent 40, 35 and 28 min less grazing per day at peak, mid, and late lactation, respectively, and the cows receiving 6 kg/DM/day spent 83, 74 and 84 min less grazing per day at peak, mid, and late lactation, respectively, when compared with unsupplemented cows.

Concentrate supplementation also affected the diurnal pattern of grazing behaviour. However, this effect was different following a.m. and p.m. feeding episodes. Following the a.m. milking there was a linear reduction in the duration of the primary grazing bout with increasing concentrate supplementation Table 3.2. However, length of the primary grazing bout following p.m. milking was not affected by concentrate supplementation, except during mid-lactation¸ when it declined linearly with increasing supplementation (Table 3.2). There was a stimulus to cease grazing at sunset at all stages of lactation, even though there was up to a 2.5 h difference in the timing of sunset (Figure 3.1). There was a linear decline in time spent grazing during darkness with increasing concentrate supplementation during early and late lactation (Period 3 and 4; Table 3.2) and mid lactation (Period 4; Table 3.2).

Table 3.1 Pasture and total DMI (kg DM/day), milk and component yield (kg/day) and milk composition (%) during peak, mid, and late lactation for New Zealand (NZ) and North American (NA) cows offered 0, 3, or 6 kg DM/day concentrates.

	Strain		Diet			SED		*P*-value	
	NZ	NA	0	3	6	Strain	Diet	Strain	Diet
Peak Lactation									
Pasture DMI	15.59	16.52	17.19	15.94	15.04	0.708	0.804	0.22	0.03
Total DMI	18.39	19.34	17.25	18.81	20.55	0.695	0.804	0.16	<0.001
Milk Yield	25.54	29.43	24.95	28.41	29.10	0.852	1.033	<0.001	<0.001
Fat Yield	1.06	1.16	1.08	1.19	1.07	0.052	0.068	0.05	0.12
Protein Yield	0.88	1.00	0.84	0.97	1.00	0.028	0.035	<0.001	<0.001
Lactose Yield	1.26	1.45	1.21	1.40	1.44	0.044	0.052	<0.001	<0.001
Fat %	4.18	3.97	4.29	4.20	3.74	0.159	0.182	0.17	<0.01
Protein %	3.46	3.40	3.38	3.44	3.47	0.059	0.056	0.29	0.25
Lactose %	4.93	4.92	4.87	4.93	4.97	0.044	0.028	0.86	<0.01
Mid Lactation									
Pasture DMI	15.25	18.60	18.51	16.59	15.67	0.669	0.662	<0.001	<0.001
Total DMI	18.09	21.49	18.63	19.48	21.26	0.656	0.684	<0.001	<0.001
Milk Yield	20.77	25.20	19.71	24.04	25.22	0.667	0.728	<0.001	<0.001
Fat Yield	0.86	0.92	0.79	0.94	0.94	0.031	0.031	0.05	<0.001
Protein Yield	0.73	0.86	0.68	0.82	0.88	0.021	0.022	<0.001	<0.001
Lactose Yield	1.01	1.23	0.95	1.18	1.23	0.034	0.037	<0.001	<0.001
Fat %	4.21	3.66	4.07	3.96	3.77	0.128	0.109	<0.001	<0.05
Protein %	3.55	3.41	3.47	3.43	3.54	0.048	0.042	<0.01	<0.05
Lactose %	4.88	4.88	4.85	4.89	4.91	0.038	0.034	0.87	0.26
Late Lactation									
Pasture DMI	15.24	18.40	17.77	16.81	15.88	0.604	0.685	<0.001	<0.05
Total DMI	18.13	21.17	17.86	19.51	21.58	0.588	0.673	<0.001	<0.001
Milk Yield	15.90	19.54	14.61	18.74	19.81	0.713	0.782	<0.001	<0.001
Fat Yield	0.78	0.85	0.72	0.85	0.87	0.034	0.036	<0.05	<0.001
Protein Yield	0.64	0.77	0.59	0.74	0.78	0.024	0.027	<0.001	<0.001
Lactose Yield	0.76	0.94	0.69	0.90	0.96	0.037	0.040	<0.001	<0.001
Fat %	4.95	4.38	5.01	4.59	4.41	0.155	0.114	<0.001	<0.001
Protein %	4.06	3.94	4.09	3.94	3.98	0.074	0.063	0.11	0.06
Lactose %	4.74	4.81	4.72	4.80	4.82	0.124	0.072	0.12	0.07

Table 3.2 Summary of grazing and ruminating behaviour during peak, mid, and late lactation for New Zealand (NZ) and North American (NA) cows offered 0, 3, or 6 kg DM/day concentrates. Daily grazing and ruminating time, time spent grazing during four distinct periods of the 24 h period, and length of first grazing bout following a.m. and p.m. concentrate allocation.

	Total Time Available (Min)	Strain		Diet			SED		*P*-value	
		NZ	NA	0	3	6	Strain	Diet	Strain	Diet
Peak lactation										
Total grazing time[1]	1270	444	420	473	433	390	10.5	12.5	<0.01	<0.001
Total ruminating time[1]	1270	541	538	513	541	565	10.4	10.2	0.83	<0.001
Period 1[2] grazing	340	161	157	175	155	147	5.4	7.1	0.53	<0.001
Period 2[2] grazing	260	168	161	172	163	158	4.4	5.8	0.12	0.07
Period 3[2] grazing	260	67	57	71	66	50	3.9	4.9	<0.05	<0.001
Period 4[2] grazing	410	49	44	57	49	34	3.7	4.9	0.22	<0.001
a.m. grazing bout[3]		103	104	108	104	98	4.9	5.5	0.83	0.18
p.m. grazing bout[4]		73	76	80	74	68	7.4	5.8	0.54	0.31
Mid Lactation										
Total grazing time[1]	1250	446	427	473	438	399	8.9	10.2	0.022	<0.001
Total ruminating time[1]	1250	421	433	417	435	429	8.7	8.6	0.18	0.01
Period 1[2] grazing	360	172	171	183	170	161	4.7	5.4	0.86	<0.001
Period 2[2] grazing	290	207	201	222	205	186	4.6	5.5	0.16	<0.001
Period 3[2] grazing	200	15	12	14	14	12	2.8	2.6	0.23	0.60
Period 4[2] grazing	400	52	44	54	49	40	4.8	4.8	0.09	<0.01
a.m. grazing bout[3]		140	150	154	147	134	4.9	5.6	<0.05	<0.01
p.m. grazing bout[4]		98	104	125	98	80	8.3	10.5	0.47	<0.001
Late Lactation										
Total grazing time[1]	1330	480	462	508	480	424	11.5	13.5	0.44	<0.001
Total ruminating time[1]	1330	414	432	424	430	414	9.6	11.5	0.15	0.49
Period 1[2] grazing	400	209	213	232	215	185	7.2	7.2	0.62	<0.001
Period 2[2] grazing	170	159	157	157	160	157	2.0	2.2	0.62	0.24
Period 3[2] grazing	350	65	56	74	60	48	5.3	5.6	0.07	<0.001
Period 4[2] grazing	410	47	36	45	44	34	4.1	4.9	<0.01	<0.05
a.m. grazing bout[3]		162	168	175	167	153	5.3	5.7	0.29	<0.001
p.m. grazing bout[4]		186	178	183	184	179	5.3	6.5	0.11	0.75

[1]Total time spent grazing or ruminating does not include time cows off pasture, at milking.
[2]Period 1: Post a.m. milking to p.m. milking, Period 2: Post p.m. milking to sunset, Period 3:post-sunset to 23:50 h, Period 4: 00:00 h to a.m. milking.
[3]Duration of primary grazing bout after a.m. concentrate allocation.
[4]Duration of primary grazing bout after p.m. concentrate allocation.

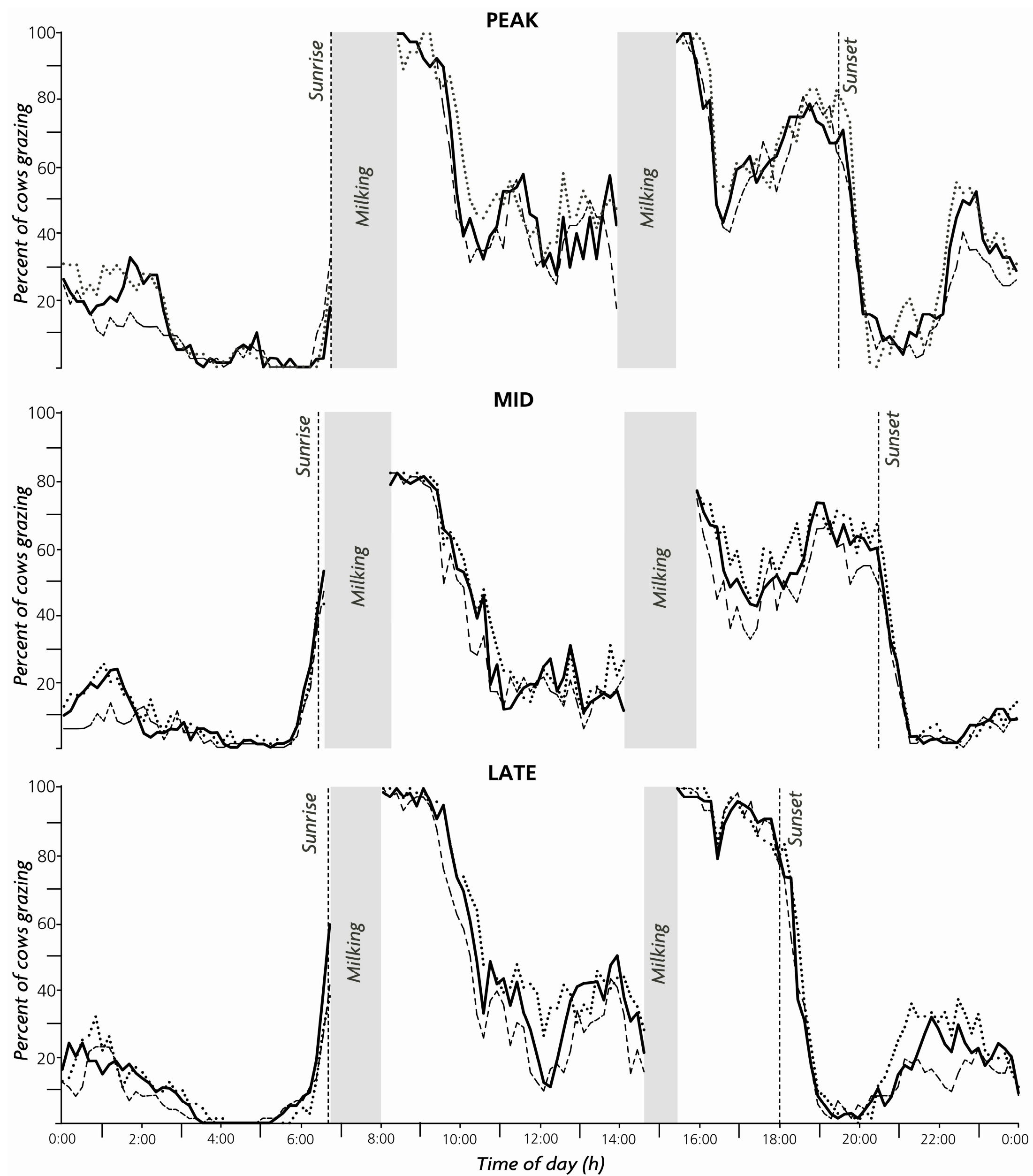


Figure 3.1 Diurnal profile of cows grazing during peak, mid, and late lactation when offered 0, 3 or 6 kg DM/day concentrate (0 kg DM/day = dotted line, 3 kg DM/day = solid line and 6 kg DM/day = dashed line). Shading represents milking time, and vertical dashed lines represent sunrise and sunset.

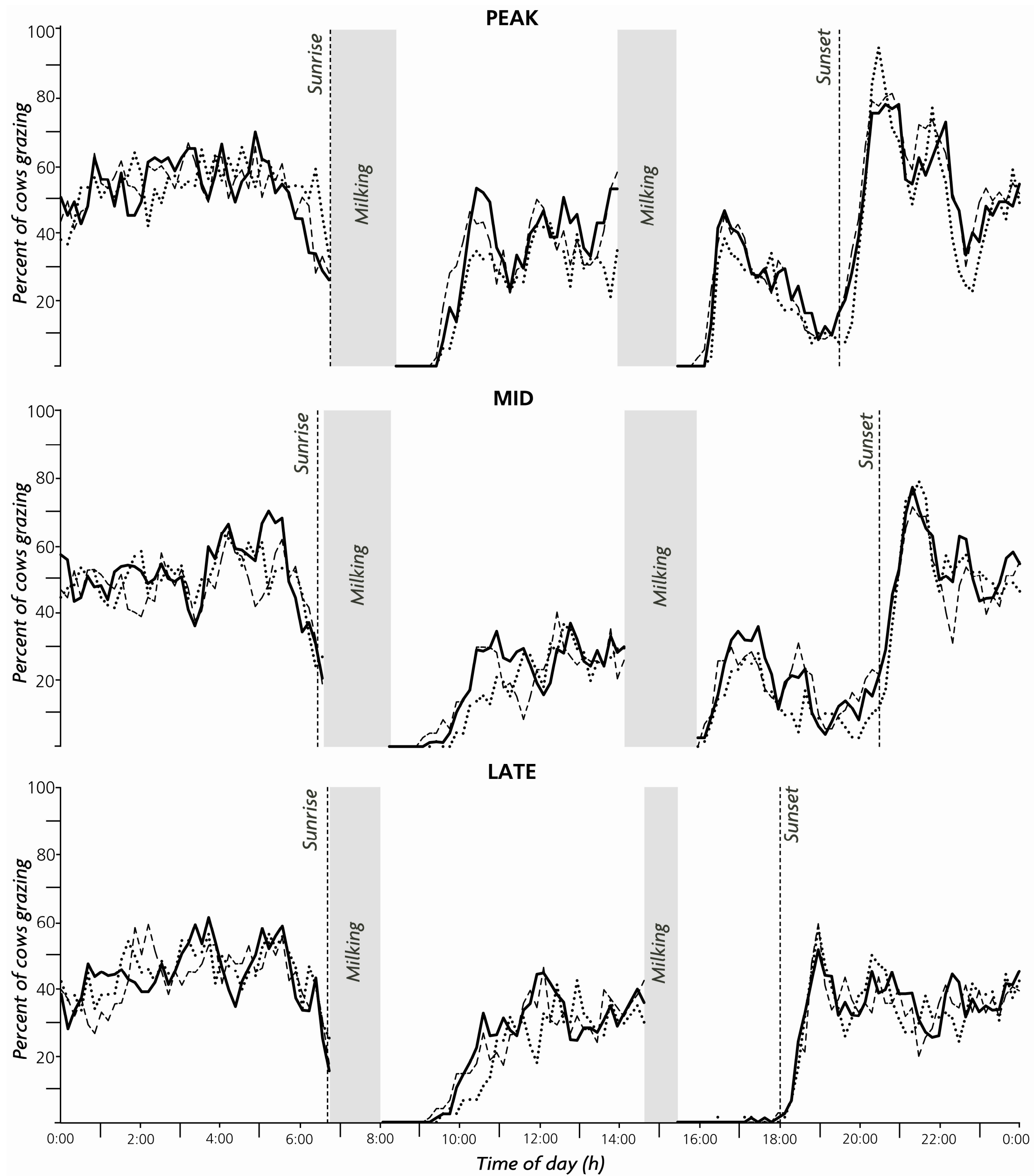


Figure 3.2 Diurnal profile of rumination in cows during peak, mid, and late lactation when offered 0, 3 or 6 kg DM/day concentrate (0 kg DM/day = dotted line, 3 kg DM/day = solid line and 6 kg DM/day = dashed line). Shading represents milking time, and vertical dashed lines represent sunrise and sunset.

3.5 Discussion

The objective of this study was to examine the grazing behaviour of cows differing in their genetic ancestry and consuming different amount of concentrates. Although previous studies have reported average daily behaviour data, highlighting total daily time spent grazing and ruminating, the current study investigated the diurnal patterns of grazing behaviour, thereby providing a greater understanding of when supplementation affects the diurnal profile.

Milk production and pasture DMI data are consistent with previous studies comparing similar genetic strains (Kolver et al., 2002; Kennedy et al., 2003; Horan et al., 2004; Linnane et al., 2004). North American cows grazed for less time but had greater pasture and total DMI in mid and late lactation than their NZ counterparts. The difference in pasture DMI, therefore, reflects the greater average bite mass by NA cows. Of particular note was the similar pasture DMI of NA and NZ cows in early lactation, even though the NA cows were producing 13% more milk. Data presented indicate that NZ cows reach peak DMI earlier than NA cows and these results are consistent with the reported predisposition of NA cows to lose more BCS in early lactation (Roche et al., 2006a; McCarthy et al., 2007a) indicating the low partition of the concentrate energy for maintenance (Bargo et al., 2002).

Previously it has been reported that increasing the amount of supplement negatively affects pasture DMI, when pasture is not limited (Stockdale, 2000b; Gekara et al., 2001; Kennedy et al., 2003; Horan et al., 2004). It has also been demonstrated that SR is poorly related to level of concentrate feeding, instead reflecting the energy balance of cows not being supplemented (Delaby et al., 2001). The decreased energy balance in high genetic merit cows during early lactation is primarily the result of genetically controlled energy partitioning rather than the result of pasture intake not keeping up with milk yield (Horan et al., 2004; Roche et al., 2009). These results suggest that SR should not increase with increasing supplement either in high genetic merit cows or in cows where energy balance is not improved through supplementation. As the NA cows produced more milk and were in greater negative energy balance in early lactation, a lower SR was predicted. Results from the current study confirm this

with SR lower in NA cows than NZ cows when consuming concentrate, providing further support that substitution is lower in high yielding cows when energy requirements are not being met (Dixon and Stockdale, 1999; Bargo et al., 2003; Horan et al., 2004). Distinct grazing bouts were evident within the day, with grazing occurring predominantly during daylight hours, as has been reported previously (Hafez, 1969; Krysl and Hess, 1993). However, despite this predominate diurnal pattern; grazing did occur during darkness with an average of 52, 47 and 36 min spent grazing in the 0, 3 and 6 kg DM/day groups, respectively. Grazing behaviour data indicate that the major grazing bouts follow a.m. and p.m. milking, with a greater amount of time spent grazing between p.m. and a.m. milking, as was reported by Soriano et al. (2000) and Scaglia et al. (2009), probably because of the greater amount of time available.

The grazing profile indicated a linear decline in grazing time with increasing concentrates in the primary grazing bout following a.m. milking, consistent with the reduced pasture DMI. In comparison, there was no association between length of time in the primary grazing bout following p.m. milking and amount of concentrate offered, with the exception of mid lactation, indicating that different factors appear to interrupt grazing following a.m. and p.m. feeding.

If one were to assume that less than 50% of pasture DMI occurred between a.m. and p.m. milking, as reflected by the grazing profile, cows consumed less than 8 kg DM pasture following a.m. milking. This is approximately 50-60 kg fresh pasture, well below rumen capacity, and suggests that rumen fill is unlikely to be the factor responsible for interrupting grazing between a.m. and p.m. milking. A plausible explanation for the cessation of grazing following a.m. milking could, therefore, be neuroendocrine factors secreted in response to the presence of food in the digestive tract or to products of digestion; for example, propionate, as reported by Faverdin (1999), is implicated as a satiety factor. A number of neuroendocrine proteins have been associated with a hunger or satiety role, their release coincident with the beginning or cessation of a meal (Roche et al., 2008a). For example, Roche et al. (2007b) reported circulating ghrelin concentrations before feeding were not affected by concentrate supplementation, but increasing supplementation at the a.m. milking was associated with a linear decline in postprandial ghrelin concentrations, thereby offering a potential

neuroendocrine basis for reduced grazing time and the subsequent reduction in pasture DMI when supplements are offered. Although Roche et al. (2007b) investigated only one peptide associated with feed intake; it provides a possible explanation for why supplemented cows ceased grazing earlier than unsupplemented cows in a dose–dependent way in this study. The lack of information for ruminants on peptides associated with feed intake in monogastric species warrants further work.

In contrast to the profile of grazing behaviour following the a.m. grazing episode, data indicate cessation of grazing in the p.m. may be a photoperiod effect, with sunset as the signal to cease grazing, regardless of supplementation, stage of lactation, or, interestingly, timing of sunset. If neuroendocrine factors did result in a cessation of grazing between a.m. and p.m. milking, results indicate that the anticipated arrival of darkness overrides these signals following p.m. supplementation. This is supported by Gibb et al. (1998) and Rutter et al. (2002a), who reported that cows reduce mastication time, increase bite rate, and, thereby, increase pasture DMI later in the p.m.

Other authors (Taweel et al., 2004; Gregorini et al., 2006), have reported that rumen fill signals grazing to cease following the p.m. feed. This may be true when day length is long, as was the case in the study by Taweel et al. (2004), with the dusk grazing bout ceasing at 2200 h, allowing cows to achieve rumen fill. However, it is not the reason for the cessation of grazing in early or late lactation in the study reported here, when day length was short. Nevertheless, the observation that rumen fill was coincident with the end of the p.m. grazing bout during long day periods could help explain why concentrates reduced the length of the primary p.m. grazing bout during mid-lactation but not at peak or late lactation. During mid lactation, time from p.m. milking to sunset was 4.7 h compared with 4.2 and 2.7 at peak and late lactation, respectively, thereby, providing the cow with sufficient time to reach rumen fill before sunset. Whereas during late lactation, when day length was shortest, cows adjusted their grazing behaviour by grazing for longer in the a.m., as if instinctively aware of the shorter period for grazing following the p.m. milking. This compensation in grazing behaviour during times of shorter day length is further evidence that DMI is regulated physiologically from energy balance signals, and is only limited by rumen fill during the p.m. feed during long day cycles. Further evidence for the physiological regulation

of DMI in dairy cows is the linear decline in grazing during darkness associated with increasing amounts of supplementation. Results from this study indicate a possible satiety effect from the products of digestion from supplementation, with resulting differences in time spent grazing and pasture DMI, when supplements are offered to grazing dairy cows.

3.6 Conclusions

Grazing behaviour is affected by cow genetics, day length and the feeding of concentrates, with grazing prioritized more in the p.m. before impending darkness than in the a.m. after milking. Sunrise and sunset are major stimuli for the beginning and cessation of grazing, respectively. Physiological factors appear to interrupt a.m. grazing and this occurs earlier in cows fed supplements. Further research is required to determine the physiological factors regulating this satiety response to supplements.

Chapter 4
Timing of Supplementation Alters Grazing Behaviour and Milk Production Response in Dairy Cows.

4.1 Abstract

Offering feed supplements to grazing dairy cows results in substitution of pasture; however, previous data indicate that the time at which concentrate supplements are offered might affect the level of substitution. These data indicated that cows grazed more intensely pre-sunset, regardless of the amount of supplement offered. It was, therefore, hypothesized that substitution rate would be less, and response to supplement greater if cows received their supplement in the p.m. rather than the a.m. Forty eight multiparous, non-pregnant, Holstein-Friesian cows, approximately 60 days in milk, were randomly allocated to one of three treatments in an incomplete crossover arrangement. Treatments were: pasture only (PASTURE), pasture + 3 kg dry matter (DM) concentrate supplement offered during a.m. milking (AMSUP), and pasture + 3 kg DM concentrate supplement offered during p.m. milking (PMSUP). Time spent grazing and calculated pasture dry matter intake did not differ between the AMSUP and PMSUP cows. However, there was a tendency (0.18 kg milk/kg concentrate DM; $P < 0.1$) for an increased marginal milk response (kg milk/kg DM supplement) for the AMSUP cows when compared with PMSUP cows. Irrespective of when supplements were offered, supplementation reduced total grazing time by a similar amount, and the reduction in time spent grazing was evident throughout the day. Cows in the PMSUP group ruminated for longer and cows in the AMSUP group spent more time idle compared with the PASTURE groups. Cows in the AMSUP group grazed for less time during the major a.m. grazing bout following a.m. milking compared with PMSUP cows; in comparison, the major p.m. grazing bout following p.m. milking was unaffected by supplementation. Results indicate possible improvements in marginal milk response to supplements from altering the timing of delivery.

4.2 Introduction

Low DMI is a major limitation to milk production in pasture-based dairy systems resulting in nutrient intakes that are insufficient to match the milk production potential of the grazing dairy cow (Kolver and Muller, 1998). In an attempt to increase total DM and ME intakes, supplements may be offered to grazing cows. However, the marginal milk response (MR) to supplements varies markedly, primarily because of a reduction in pasture DMI (Stockdale, 2000; Bargo et al., 2003). The decrease in pasture DMI with increasing supplement DMI is termed substitution (Bargo et al., 2003).

Substitution is reflected by changes in the dairy cows grazing behaviour, with a reported 12 min decrease in grazing time for every 1 kg DM supplement consumed (Bargo et al., 2003; Sheahan et al., 2011) and an increase in rumination time (Sheahan et al., 2011). Sheahan et al. (2011) reported that when cows were supplemented at a.m. and p.m. milking the effect on grazing time was not consistent throughout the day, with supplementation reducing grazing time during the day but not immediately preceding sunset, which is the most intensive grazing bout of the day (Gibb et al., 1998; Scaglia et al., 2009). Sheahan et al. (2011) hypothesized that the cessation of grazing following a.m. supplementation was due to neuro-endocrine factors resulting from the digestion of feed, rather than physical factors, and that sunset signalled the end of grazing following p.m. milking, irrespective of supplementation in the p.m.

Based on the lack of effect of concentrate supplementation on time spent grazing before sunset for cows supplemented at a.m. and p.m., it was hypothesized that substitution would be less, and therefore, the milk production response to concentrate greater, when cows received the same amount of concentrate supplement in the p.m. rather than a.m.

4.3 Materials and Methods

This experiment was conducted at Lye Farm, DairyNZ, Hamilton, New Zealand from September to October 2011, and was approved by the Ruakura Animal Ethics Committee, Hamilton, New Zealand.

4.3.1 Experimental design

The experimental design was an incomplete cross-over arrangement, with 48 multiparous, non-pregnant, Holstein-Friesian cows, approximately 60 days in milk, randomly assigned to one of three treatments (n = 16 cows/treatment): pasture only (**PASTURE**), pasture plus 3 kg DM concentrate supplement offered during a.m. milking (**AMSUP**), and pasture plus 3 kg DM concentrate supplement offered during p.m. milking (**PMSUP**). The initial concentrate supplement allocation was 1 kg DM/day, and then increased by 1 kg DM/day until full allocation was obtained. Cows then underwent a 10 d adaptation period before a 7 d measurement period. At the end of the 7 d measurement period cows were reassigned to a new treatment in the following manner: of cows previously in AMSUP treatment group, eight were assigned to PMSUP and eight to PASTURE treatment; of cows previously in PMSUP treatment group, eight were assigned to AMSUP treatment, and eight to PASTURE treatment; of cows previously in PASTURE treatment group, eight were assigned to AMSUP treatment and eight to PMSUP treatment. A 10 d adaptation period followed by a 7 d measurement period was repeated.

4.3.2 Pasture and Concentrate Supplement

Cows were grazed as one herd for 24 h/day for the duration of the experiment and were only removed for a.m. and p.m. milking. Pasture allowance was sufficient to ensure DMI up to approximately 20 kg DM/day for the unsupplemented cows. Cows had access to a fresh allocation of pasture after both a.m. and p.m. milking. Each allocation was 50% of the daily allowance. Pasture was of high quality (Table 4.1) throughout the experiment and each pasture allocation was sampled by hand 'plucking' a representative pasture sample to simulate grazing, just prior to cows entering the paddock, for quality analysis (Dairy One, Ithaca, USA). The concentrate supplement was offered (3 kg DM) during either the a.m. or p.m. milking, in accordance with allocated treatment. The supplement was a pelleted concentrate composed of distillers grain (35% DM), palm kernel expeller (25% DM), maize grain (15% DM), wheat middlings (15% DM), and minerals (10% DM). Refusals were recorded daily with an

average of 2.90 kg DM and 2.96 kg DM consumed (SED 0.030, $P < 0.05$) for the AMSUP and PMSUP cows, respectively.

Table 4.1 Chemical composition of bulked a.m. and p.m. pasture samples and concentrate supplement offered.

	a.m. Pasture[1]	p.m. Pasture[1]	Supplement
DM %	12.8	18.4	93.5
	% of DM		
CP	27.0	26.5	21.8
ADF	23.9	23.8	22.3
NDF	44.0	43.1	35.7
Lignin	2.4	2.4	5.2
NFC[2]	22.8	24.3	34.5
Starch	0.6	0.6	14.3
Fat	4.3	4.2	5.65
Ash	9.9	9.8	10.5
IVTD[3] 24hr % DM	90.8	90.8	75.5
NDFD[4] 24hr % of NDF	79.5	78.5	31.5
ME MJ/Kg DM[5]	13.9	13.9	10.3

[1]Pasture samples were collected immediately prior to cows grazing fresh pasture allocation.

[2]Non-fibre carbohydrate (NFC)

[3] *in-vitro* true digestibility (IVTD)

[4] Neutral detergent fibre digestibility (NDFD)

[5]ME calculated from IVTD (IVTD x 0.172-1.707) (CSIRO, 2007)

4.3.3 Pasture Dry Matter Intake

Pasture DMI was calculated from mean daily milk energy output plus cow maintenance requirements for BW change. Cows were weighed once a week after a.m. milking. Bodyweight gain/loss was calculated for each individual cow by calculating the difference in BW over the 4 wk period. The efficiency with which energy was used for milk production was assumed to be 65%, and the maintenance requirements for lactating grazing dairy cows was 0.6 MJ/kg BW 0.75 (Holmes et al., 2003). The energy required for 1 kg BW gain or supplied from 1 kg BW loss was assumed to be 32 and 25 MJ, respectively (Holmes et al, 2003). Energy intake was divided by the mean pasture ME concentration to calculate DMI and pasture DMI was calculated from total DMI minus concentrate intake.

4.3.4 Milk Production

Milking times were 0700 h and 1600 h. Individual a.m. and p.m. milk yields were recorded daily (Westfalia Surge, Oelde, Germany) during the entire experiment, but only individual milk weights during the 7 d measurement periods were used for analyses. Separate a.m. and p.m. milk samples were collected on 2 d within both 7 d measurement periods for milk composition analysis. Milk fat, CP, casein and lactose concentrations were determined by Fourier-transform infrared spectroscopy (FT120, Foss Electric, Denmark) on separate p.m. and a.m. samples.

4.3.5 Grazing Behaviour

Grazing behaviour was recorded for 24 h during both 7 d measurements periods. Time spent grazing, ruminating (lying and standing), and idle (not grazing or ruminating) was determined by recording each cow's activity at 10 min intervals throughout a 24 h period while the cows were at pasture (Gary et al., 1970). Grazing for this experiment was defined as 'cows in the act of eating'. The 24 h observations were further divided into four time blocks to determine the effect of treatment on cow grazing behaviour at defined times during the day. The four time blocks were: Time block (**TB**) 1 (post a.m. milking to p.m. milking), TB 2 (post p.m. milking to sunset), TB 3 (post

sunset to 2350 h), and TB 4 (0000 h to a.m. milking). The duration of the major grazing bout after each milking was calculated as the difference between the time each cow entered the paddock and the time immediately prior to the recording of two consecutive non-grazing events (either ruminating or idle).

4.3.6 Statistical Analysis

Average a.m., p.m., and daily milk yields were calculated for each cow during each of the two measurement periods. Additionally, average fat, protein, casein, and lactose composition and yields were also calculated for each cow during the two measurement periods. The total time spent in a particular grazing behaviour (grazing, ruminating or ide) was calculated for each cow in each measurement period and during the defined time blocks and major grazing bout after a.m. and p.m. milking. Each of these calculated variables was then analysed with GenStat 14.1 (VSN International, Hemel Hempstead), using mixed models including measurement period, treatment, interaction of measurement period with treatment as fixed effects and cow and measurement period within cow as random effects. Equal variances were used for the two measurement periods.

4.4 Results

The effects of a.m. or p.m. supplementation on milk production are presented in Table 4.2. Cows in the AMSUP and PMSUP groups produced more ($P < 0.001$) milk and milk components (kg/cow per d) than cows fed pasture alone. There was a tendency ($P < 0.07$) for the AMSUP cows to produce more milk (kg/cow per d) than the PMSUP cows. Cows in the AMSUP group produced more ($P < 0.001$) milk and milk components at a.m. milking than PASTURE and PMSUP groups, which did not differ from each other. Similarly, cows in the PMSUP produced more ($P < 0.001$) milk and milk components at p.m. milking than the PASTURE and AMSUP groups, which did not differ from each other. Marginal MR to supplements for the AMSUP and PMSUP groups were 0.52 and 0.34 kg milk/kg DM supplement, respectively. Supplementation resulted in a reduction in calculated pasture DMI (Table 4.2). PASTURE cows

consumed more pasture than AMSUP and PMSUP groups, which did not differ from each other.

Table 4.2 Summary of milk production, milk components and estimated pasture intake for cows on pasture only (PASTURE), pasture + a.m. supplement only (AMSUP), and pasture + p.m. supplement only (PMSUP).

	PASTURE	AMSUP	PMSUP	SED	*P* < value
Daily					
Pasture DMI(kg/d)[1]	16.8	15.1	14.9	0.151	< 0.001
Milk Yield (kg/d)	26.6	28.1	27.6	0.248	< 0.001
Fat %	4.46	4.38	4.38	0.100	0.66
Crude Protein %	3.63	3.63	3.64	0.017	0.74
Casein %	2.85	2.85	2.86	0.017	0.97
Lactose %	4.76	4.77	4.74	0.019	0.27
Fat (kg/d)	1.18	1.22	1.20	0.029	0.32
CP (kg/d)	0.96	1.01	1.00	0.010	< 0.001
Casein (kg/d)	0.75	0.80	0.78	0.008	< 0.001
Lactose (kg/d)	1.27	1.34	1.31	0.014	< 0.001
a.m.					
Milk Yield (kg)	17.4	18.9	17.2	0.291	< 0.001
Fat %	3.87	4.06	3.41	0.166	< 0.001
Crude Protein %	3.60	3.59	3.62	0.018	0.25
Casein %	2.81	2.80	2.81	0.019	0.84
Lactose %	4.75	4.75	4.74	0.019	0.73
Fat (kg)	0.67	0.77	0.58	0.038	< 0.001
CP (kg)	0.62	0.67	0.62	0.010	< 0.001
Casein (kg)	0.48	0.52	0.48	0.008	< 0.001
Lactose (kg)	0.82	0.89	0.81	0.014	< 0.001
p.m.					
Milk Yield (kg)	9.3	9.2	10.4	0.250	< 0.001
Fat %	5.56	5.06	5.92	0.111	< 0.001
Crude Protein %	3.69	3.73	3.68	0.019	< 0.05
Casein %	2.93	2.96	2.93	0.019	0.230
Lactose %	4.78	4.80	4.74	0.026	0.11
Fat (kg)	0.52	0.46	0.61	0.022	< 0.001
CP (kg)	0.34	0.34	0.38	0.008	< 0.001
Casein (kg)	0.27	0.27	0.30	0.007	< 0.001
Lactose (kg)	0.44	0.44	0.49	0.012	< 0.001

[1] Pasture DMI was calculated from milk production.

Summarised grazing behaviour data are presented in Table 4.3. There was an effect of treatment on the total time spent grazing, PASTURE cows grazed for 31 and 37 min longer ($P < 0.01$), than the AMSUP and PMSUP cows, respectively; this reflected a reduction in time spent grazing of 11 and 13 min/kg supplement. There was no effect of timing of supplementation on time spent grazing during the defined time blocks throughout the day, with the exception of TB 4, when PMSUP cows grazed for less ($P < 0.05$) time than the PASTURE cows. During the hours of darkness (TB 3 and 4), PASTURE cows grazed for longer ($P < 0.05$) than AMSUP and PMSUP cows, 18 and 22 min respectively. Cows in the AMSUP group had a shorter ($P < 0.05$) major grazing bout following a.m. milking than PMSUP cows. In comparison, there was no effect of treatment on the major grazing bout following p.m. milking.

Timing of supplementation altered total time spent ruminating, with cows in the PMSUP treatment ruminating for 24 and 28 min more ($P < 0.05$) than PASTURE and AMSUP groups, respectively; the greatest differences occurred during the hours of darkness (TB 3 and 4). No cows from any treatment were recorded ruminating in the period between p.m. milking and sunset (TB 2). There was also an effect of treatment on the total time spent idling with cows in the AMSUP treatment idle for 43 and 22 min more ($P < 0.01$) than PASTURE and PMSUP treatments, respectively.

Table 4.3 Summary of grazing, ruminating and idling behaviour. Daily total grazing, ruminating and idling times, time spent grazing, ruminating and idling during four time blocks (TB) during a 24 h period, and length of major grazing bout following a.m. and p.m. milking for cows on pasture only (PASTURE), pasture + a.m. supplement only (AMSUP), and pasture + p.m. supplement only (PMSUP).

	Total Time Available (Min)	PASTURE	AMSUP	PMSUP.	SED[4]	P-Value
Total Grazing Time[1]	1280	541	510	504	11.2	<0.01
Time block 1[2]	425	273	263	264	7.7	0.31
Time block 2[2]	140	128	127	123	3.0	0.34
Time block 3[2]	305	93	83	88	6.8	0.36
Time block 4[2]	410	46	38	29*	7.9	0.10
Hours of darkness (TB3 and TB4)		139	121	117	9.3	0.05
a.m. major grazing bout[3]		143	128‡	155	11.6	0.06
p.m. major grazing bout[3]		153	151	145	6.2	0.46
Total Ruminating Time	1280	344	340	368	9.7	<0.05
Time block 1[2]	425	68	68	66	5.2	0.91
Time block 2[2]	140	0	0	0		
Time block 3[2]	305	97	97	106	5.2	0.15
Time block 4[2]	410	180	178	193	8.0	0.13
Hours of darkness (TB3 and TB4)		277	274	299	9.1	<0.05
Total Idle Time[1]	1280	366	409	387	13.4	<0.01
Time block 1[2]	425	71	87	84	6.5	<0.05
Time block 2[2]	140	10	12	13	2.9	0.55
Time block 3[2]	305	110	121	110	7.9	0.32
Time block 4[2]	410	174	188	181	7.8	0.24
Hours of darkness (TB3 and TB4)		308	290	284	12.0	0.13

[1]Total time spent grazing, ruminating or idle does not include time cows off pasture for milking

[2]TB 1: Post a.m. milking to p.m. milking, TB 2: Post p.m. milking to sunset, TB 3: Post sunset to 23:50h, TB 4: 00:00h to a.m. milking.

[3]Duration of major grazing bout after a.m. and p.m. milking (min), calculated as the difference between the time cows entered the paddock and the time immediately prior to the recoding of two consecutive non-grazing events.

[4]Standard error of the difference (SED)

* indicates difference ($P < 0.05$) between PMSUP and PASTURE groups.

‡ indicates difference ($P < 0.05$) between AMSUP and PMSUP groups

4.5 Discussion

The objective of this experiment was to determine if previously reported differences in the effects of supplementation at a.m. and p.m. milking on grazing behaviour (Sheahan et al., 2011) could be exploited to minimize pasture substitution and increase marginal MR to supplements. In the present experiment, total grazing time did not differ between AMSUP and PMSUP cows. However, there was a tendency for an increased marginal MR when cows were supplemented in the a.m. rather than the p.m. The timing of supplementation did not affect calculated DMI, with results indicating a sustained decrease in grazing time, regardless of timing of supplementation.

Supplementing grazing cows in either the a.m. or the p.m. similarly, reduced total time spent grazing compared with PASTURE cows. The decline in time spent grazing (11-13 min/kg DM supplement) is consistent with the 12 min/kg DM supplement reported by Bargo et al. (2003) and Sheahan et al. (2011). However, the lack of effect on total time spent grazing when supplementing in the p.m. rather than the a.m. is contrary to the proposed hypothesis. This result was unexpected particularly because the grazing behaviour was consistent with the concept that neither a.m. nor p.m. supplementation affected grazing time between p.m. supplementation and sunset, but a.m. supplementation reduced time spent grazing in the major a.m. grazing bout. Consistent with Sheahan et al. (2011), grazing during the period between p.m. milking and sunset does not appear to be regulated by products of digestion or associated endocrine factors but rather appears to be regulated by impending darkness. Despite the lack of effect on total time spent grazing, data from the present experiment provide some evidence of the effects of supplementation on factors regulating DMI in grazing cows, as the difference in total grazing time was a result of an equal reduction in time spent grazing in three of the four time blocks within a 24 h period. These data are consistent with Sheahan et al. (2011), who supplemented cows at a.m. and p.m., and indicate that the effect of supplementation on grazing time is sustained well beyond the act of ingestion and likely, beyond the period of digestion.

Between a.m. and p.m. milking, grazing times were not affected by timing of supplementation; however, the length of the first a.m. grazing bout, which is one of the major grazing bouts during the day (Gibb et al., 1998; Taweel et al., 2004), was reduced in the AMSUP cows, despite less time grazing during the hours of darkness than unsupplemented cows. This result is consistent with Roche et al. (2007b) and Sheahan et al. (2011), who reported that the cessation of the major grazing bout in the a.m. is regulated by neuro-endocrine factors associated with products of digestion. As rumen pH and VFAs were not measured in the current experiment, it is not possible to determine likely associations between products of digestion and factors circulating in the blood. However, offering the concentrate supplement likely altered the ruminal acetate: propionate ratio, increasing propionate production at the expense of acetate compared with unsupplemented cows (Orskov, 1986; Bannink et al., 2008). Therefore, the reduced major a.m. grazing bout in the AMSUP cows is consistent with supplementation increasing propionate production, a factor known to increase insulin production and reduce grazing time (Oba and Allen, 2003). The lack of effect due to supplementation on the p.m. major grazing bout indicates a photoperiod effect on intake regulation that may supersede digestion-derived neuro-endocrine factors (Sheahan et al., 2011). Data, therefore, indicate different factors regulating hunger and satiety in diurnal animals.

The increased rumination time for the PMSUP cows is consistent with reports that supplementing grazing cows increased time spent ruminating (Phillips and Leaver, 1986; Sheahan et al., 2011). However, the rumination time for the AMSUP cows was similar to that of PASTURE cows, indicating that the factors regulating rumination are dependent on timing of feed. As the majority of rumination occurred during the hours of darkness, when little grazing occurred, the reduced rumination time indicates that the AMSUP did not need to further masticate their feed and, therefore, could spend more time in idle behaviour.

Increased milk production, when grazing cows are supplemented with concentrated feeds, is widely recognized (Bargo et al., 2002; Kennedy et al., 2008; Sheahan et al., 2011). Results from the current study indicate a tendency for increased marginal MR when supplements were offered in the a.m. rather than p.m. This is

contrary to Trevaskis et al. (2004) who reported an increase in milk production when supplementing grazing dairy cows in the p.m. compared with the a.m. This could be due to the allocation of daily pasture allowance once a day in their study, compared with fresh pasture allocations after a.m. and p.m. milking in the current study.

As total grazing time did not differ between supplemented treatments, the increase in milk production may be related to bite mass or bite rate rather than grazing time. Numerous studies have reported that increasing the amount of concentrate reduces grazing time but does not affect bite rate or bite mass; (see review by Bargo et al., 2003); however, bite mass data are often calculated indirectly, and this, therefore, does not take into account grazing dynamics within a grazing bout. Short term intake rate is dependent upon bite mass (Gibb, 2006), which decreases as rumen fill increases, even though bite rate is maintained (Chilibroste et al., 1997; Gregorini et al., 2007). Bite mass for the PMSUP cows may have been reduced after p.m. supplementation, despite similar grazing times, due to presence of extra feed in the rumen compared with PASTURE and AMSUP cows. If true, cows supplemented in the p.m. would have reduced their bite mass, at a time of the day when grazing is most intensive (Gibb et al., 1998, Taweel et al., 2004), and when the NSC concentration in the pasture is increased compared with in the a.m. (Orr et al., 2001). Increased milk production has been reported when cows are fed pasture with greater NSC concentration (Miller et al., 2001) or when offered their fresh allocation of pasture in the afternoon (Orr et al., 2001). These data suggest an effect of subtle changes in pasture composition on bite mass or outputs of rumen fermentation. Future work investigating the timing of supplementation will require more detailed grazing behaviour and rumen fermentation measurements, as results in the present study indicate that grazing time alone does not sufficiently explain treatment effects on milk production.

4.6 Conclusion

Supplementing grazing dairy cows at either a.m. or p.m. milking altered grazing behaviour compared with cows eating pasture alone. Data indicate that a sustained reduction in grazing time throughout the day irrespective of the time that supplements was offered. Although grazing time and calculated pasture DMI were similar in supplemented cows, there was a tendency for an increased marginal MR when cows were supplemented in the a.m. rather than the p.m. Further work investigating the products of digestion and their effect on physiological hunger and satiety factors, and how these impact grazing behaviour and milk production is warranted to understand the complexities of DMI regulation in grazing cows.

Chapter 5
Diurnal Patterns of Grazing Behaviour and Humoral Factors in Supplemented Dairy Cows.

5.1 Abstract

Offering feed supplements to grazing dairy cows' results in a reduction in grazing time. However, the effect differs depending on the time of day that feeds are offered. To understand the physiological basis for this, associations among circulating factors known to be associated with intake regulation in monogastric species and grazing behaviour in the dairy cow were investigated. Seventeen multiparous cows at 28 ± 5 days in milk grazed together and consumed 4.4 kg DM /day of a pelleted concentrate feed supplement, equally split, at a.m. and p.m. milking. Grazing behaviour was recorded over four consecutive days in all 17 cows. Blood was sampled from 10 of the 17 cows every 4 h over a 48 h period following the grazing behaviour measurements; sampling times were staggered by 2 h, to provide a diurnal profile of humoral factors. Grazing profiles illustrated major grazing bouts after a.m. and p.m. milking; however, the p.m. grazing bout was characterized as the most intensive and time spent grazing was unaffected by supplementation. Associations among proportion of cows grazing and circulating hormones and metabolites differed throughout the day. During the a.m., relationships were consistent with those reported in monogastric species, with ghrelin and non-esterified fatty acids declining and insulin increasing with feeding. In comparison, during the major grazing bout pre-dusk, ghrelin concentrations increased until sunset, despite the high proportion of cows grazing, before declining; this is consistent with ghrelin stimulating the pre-dusk grazing bout. Results indicate that humoral factors known to affect hunger and satiety in monogastric animals may also have a potential role in the physiological regulation of diurnal and feeding behaviour in ruminants.

5.2 Introduction

Relatively low DM and ME intakes are primary limitations to productivity in pasture-based dairy systems, resulting in nutrient intakes that are insufficient to match the milk production potential of the grazing dairy cow (Kolver and Muller, 1998). Supplementary feeds are often offered to grazing cows in an attempt to overcome these limitations (Stockdale, 1999; Bargo et al., 2003). However, total DMI does not increase by the amount of supplement consumed, as cows reduce their pasture DMI when offered supplementary feeds (Bargo et al., 2003; Sheahan et al., 2011). This substitution of supplement for pasture is reflected in changes in dairy cow grazing behaviour, with a reported 12 min decrease in grazing time for every 1 kg DM supplement consumed (Bargo et al., 2003; Sheahan et al., 2011).

Sheahan et al. (2011) reported that the effect of supplement on grazing behaviour was not consistent throughout the day. In cows offered a supplementary feed twice a day, grazing time during the major grazing event following sunrise was reduced, but not in the most intensive grazing event of the day immediately preceding sunset (Gibb et al., 1998). Results indicated that different factors may regulate grazing behaviour at these times.

Because of the apparent difference in the grazing behaviour response to supplementation, (Sheahan et al., 2013b) supplemented grazing dairy cows with 3 kg DM pelleted concentrate at either the a.m. or p.m. milking, hypothesizing that substitution would be less in the p.m. supplemented group. Results, however, indicated a similar reduction in time spent grazing throughout the day, regardless of when supplementary feeds were offered. These data did indicate an effect of time of day on the change in grazing behaviour in response to feeding, but irrespective of timing of feeding. Supplementary feeds caused a relatively quick reduction in grazing time in the morning, but this effect was delayed in the afternoon; these results are consistent with different factors regulating the onset and cessation of feeding in the major grazing events (i.e. post-sunrise and pre-sunset). Data also indicated an effect of supplement on grazing behaviour beyond the period of supplement consumption and possibly digestion (Sheahan et al., 2013b).

The mechanisms that regulate post-prandial satiety are still being established and this is particularly true in ruminant animals. It is believed that short-term signals, including gut hormones and neural signals from the gut, liver and higher brain centres, regulate meal initiation and termination (Murphy et al., 2006; Roche et al., 2008a). The gastrointestinal tract is the body's largest endocrine organ (Ahlman and Nilsson, 2001), releasing more than 30 known peptide hormones (Ahlman and Nilsson, 2001; Murphy et al., 2006). Although their role in the regulation of gastrointestinal function is well established, there is increasing evidence that many also influence eating behaviour (Badman and Flier, 2005; Murphy et al., 2006).

The majority of research in intake regulation has been undertaken in monogastric species. However, some of these factors are important in intake regulation in ruminant animals (Sugino et al., 2002b; Takahashi et al., 2006; Roche et al., 2007b; Roche et al., 2008a). For example, there is a linear decline in plasma ghrelin concentrations 2 h after dairy cows received a concentrate supplement in the morning (Roche et al., 2007b), coincident with the timing of grazing cessation in response to level of supplementary feeding.

The role and importance of gut-derived peptides in ruminant animals is complicated due to their unique digestive system, in that there is a constant flow of ingesta into the abomasum, as opposed to discrete meals in the monogastric. The objective of this study was to investigate circulating factors known to be associated with intake regulation in monogastric species, for a role in the regulation of grazing behaviour in the dairy cow.

5.3 Materials and Methods

This experiment was conducted at Lye Farm, DairyNZ, Hamilton, New Zealand (37º46'S 175º18'E) and all procedures were approved by the Ruakura Animal Ethics Committee, Hamilton, New Zealand.

5.3.1 Experimental design

The 17 multiparous cows used for this experiment were the control group from a larger study (Roche et al., 2008b). At the start of the experiment average cow data (mean ± standard deviation) were: calving date (17th July ± 5 d), parity (6 ± 2 yr), pre-experimental BW (481 ±61.9 kg), BCS (4.6 ± 0.38 BCS units; 10-point scale; Roche et al., 2004) and milk production (23.4 ± 3.70 kg milk/d; 1.1 ± 0.19 kg fat/d; 0.8 ± 0.13 kg protein/d; 4.6 ± 0.44 % fat; 3.4 ± 0.20 % protein).

5.3.2 Pasture management and supplementary feeds

Cows were rotationally grazed as one herd for the duration of the experiment, with the exception of when blood sampling occurred: the 10 of the 17 cows sampled were kept as a separate group for the 48-h duration of sampling for ease of blood collection. Cows had access to a fresh allocation of pasture twice daily after a.m. and p.m. milking. Pasture allowance (> 40 kg DM/cow/day) was sufficient to ensure unrestricted DMI (up to approximately 20 kg DM/day) of fresh pasture. Water was available ad libitum in each grazing area. Near Infra-Red Spectroscopy confirmed the pasture was of high quality (CP = 24.3 ± 2.40% DM; NDF = 38.4 ± 3.02 % DM; ADF = 20.4 ± 1.05 % DM; lipid = 4.1 ± 0.18 % DM; NSC = 16.8 ± 2.98 % DM; OM digestibility > 84.0 % DM; ME > 12.5 MJ/kg DM). A flat rate of 4.4 kg DM pelleted concentrate (32% crushed barley, 60% crushed maize, 2% wheat middlings, 6% molasses; CP = 14.1 ± 0.16 % DM; NDF = 16.8 ± 0.47 % DM; ADF = 7.7 ± 0.39 % DM; lipid = 3.5 ± 0.19 % DM; NSC = 56.7 ± 0.62 % DM) was split equally in two feeds daily and offered during the a.m. (0640-0800 h) and p.m. (1430-1500 h) milking.

5.3.3 Animal Measurements

5.3.3.1 Grazing Behaviour

Grazing behaviour was recorded for all 17 cows for four consecutive days. Time spent grazing, ruminating (lying and standing), and idle (not grazing or ruminating) was determined by recording each cow's activity at 10 min intervals throughout a 24 h period while the cows were in the paddock (Gary et al., 1970). Grazing was defined as

'cows in the act of eating'. The 24 h observation periods were further divided into four time blocks (**TB**): TB1 (0010-0600 h), TB2 (0610-1400 h), TB3 (1410 to 1800 h), and TB4 (1810- 0000 h). Sunrise and sunset times were averaged for the 4 d (0620 h and 1810 h, respectively: www.timeanddate.com, accessed 29 August 2012).

5.3.3.2 Jugular Catheters and Blood Sampling

Immediately following grazing behaviour measurements (the day prior to blood sampling), a jugular catheter (14 gauge x 19.6 cm; Delmed, New Brunswick, NJ) was inserted under local anesthetic into 10 of the 17 cows. After each blood collection catheters were flushed with 10 mL of isotonic saline with 50 IU/mL of sodium heparin (Mutiparin, Fisons Pharmaceuticals, NSW, Australia). Blood was sampled every 4 h for 48 h commencing at 1000 h and every 4 h thereafter until 0600 h the following morning; then the blood sampling was staggered by 2 h with collection at 0800 h and thereafter, every 4 h. The staggered blood sampling was done to minimize disruption to the cow's normal grazing behaviour by frequent removal from the paddock while still achieving 2 hourly samples relative to feeding. Cows were returned to their paddock after sampling.

5.3.3.3 Blood

Two evacuated 10 ml blood tubes, (140 IU sodium heparin and 0.117 ml of 15% K_3 EDTA) were collected from each cow. Following centrifugation (1,120 x g, 12 min, 4^oC), plasma from the EDTA-blood tubes were acidified using 0.1 N HCl, and treated with phenylmethylsufonyl fluoride (PMSF) for ghrelin analysis (as per kit instructions; Millipore, USA) and stored at -20°C until analysis. Analyses for NEFA (colorimetric method) and glucose (hexakinase method) were performed on a Hitachi 717 analyzer (Roche, Basel, Switzerland) at 30°C by Alpha Scientific Ltd., Hamilton, New Zealand. The inter- and intra- assay CV was $< 2\%$. Growth hormone (**GH**) (Downing et al., 1995), IGF-1 (Gluckman et al., 1983), insulin (Hales and Randle, 1963) and leptin (Blache et al., 2000) were measured in duplicate by double-antibody RIA with an inter- and intra- assay CV $< 6\%$. Plasma ghrelin concentrations were measured using the Millipore active ghrelin RIA kit (GHRA-88HK; Millipore Corporation, Billerica, MA).

The inter- and intra assay CV was 7% and 6%, respectively. Plasma glucagon was measured using the glucagon RIA kit (XL-85K, Millipore, USA). The kit was specific for pancreatic glucagon and cross reaction with oxynomodulin (gut glucagon) was < 0.1%. The intra assay CV of 5%. Plasma glucagon-like peptide–1 (GLP-1) was measured using a RIA kit (GLP1T-36HK, Millipore, USA), utilizing an antibody that recognizes all forms of GLP-1. The intra assay CV was 9%.

5.3.4 Statistical Analysis

A paired two-tailed t-test was performed to investigate diurnal variation in circulating humoral factors over a 24-h period. Due to the uncertain distribution of the percent time associated with behaviour observations and the repeated nature of the data per cow a bootstrap-based regression analysis with clustered regression was used to investigate the nature and significance of the behaviour and blood metabolite level association. The bootstrap regression implementation served to help isolate the regression coefficient confidence intervals and the guidelines in Efron and Tibshirani (1993) were used. The use of clustering (on cow) allowed the relaxation of the assumption of observation independence (Hamilton, 2009), by admitting common cow-based variance estimates and thus more realistically allowing the isolation of standard errors of the regression estimates.

5.4 Results

The diurnal grazing profile (Figure 5.1 a) illustrates that grazing was mainly confined to the hours between sunrise and sunset. Within this period, cows had major grazing bouts after a.m. and p.m. milking with intermittent shorter grazing events. Grazing during the hours of darkness (between sunset and sunrise) was minimal, particularly between 0200-0530 h.

Although fresh pasture was allocated after each milking, results indicate that grazing profiles were different; this was particularly evident during the first four hours after a.m. and p.m. milking, which represent when the majority of grazing occurred. Following the a.m. milking 94% of cows grazed for 1 h, from 1 h grazing gradually

declined to 35% 4 h after grazing commenced. In comparison, grazing behaviour following the p.m. milking differed in that 87% cows continued to graze for 3 h, until sunset (1800 h), at which point grazing declined to 9% by 4 h post milking.

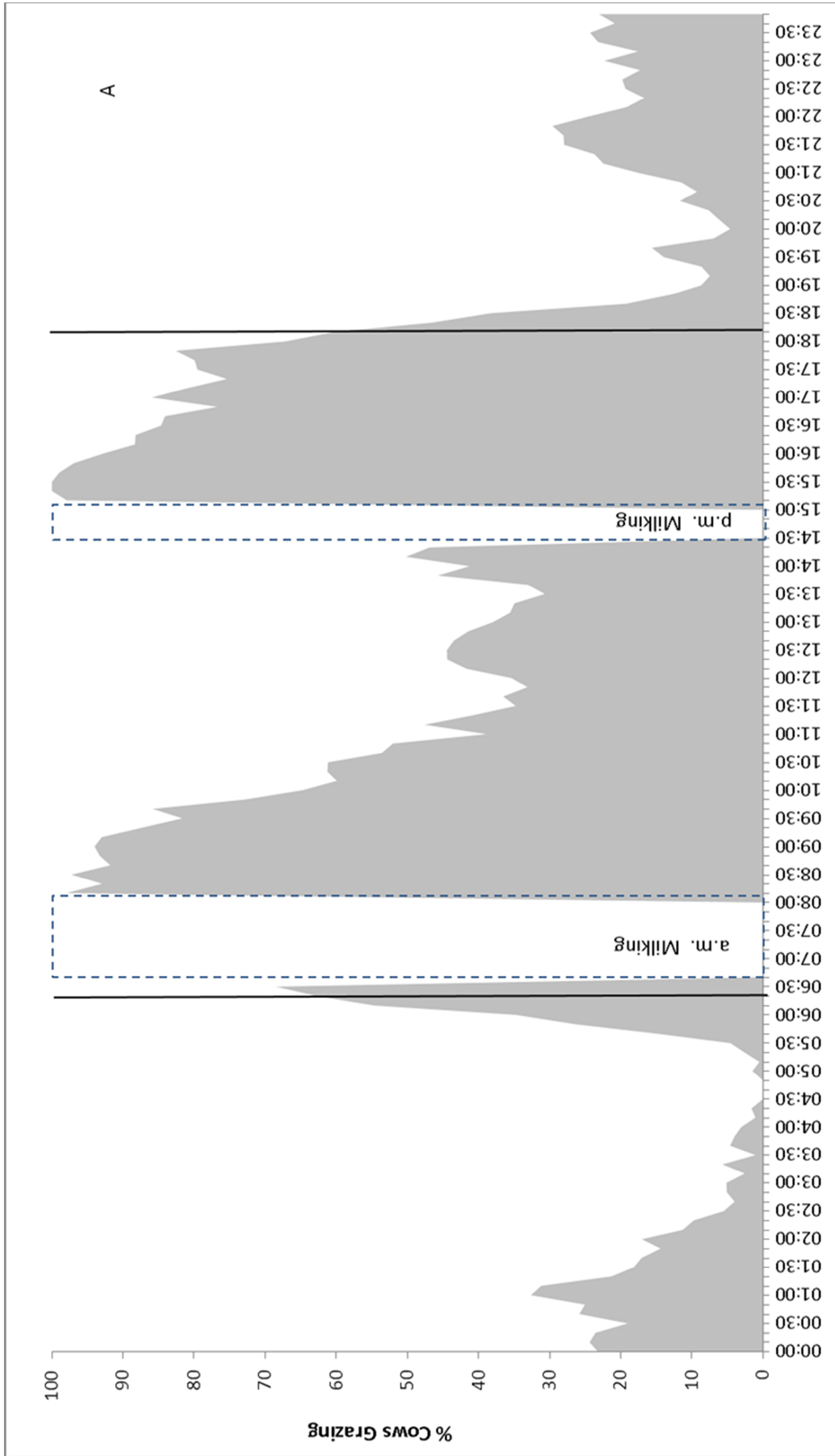
The diurnal rumination profile (Figure 5.1 b) illustrates that, although rumination occurred during the day, the majority occurred between sunset and sunrise (i.e. during darkness). The profile for the diurnal idle behaviour (Figure 5.1 c) was similar to that of the rumination profile (i.e. the majority of idle behaviour during the hours of darkness), with the exception of the period just prior to sunset, when the proportion of idle cows increased.

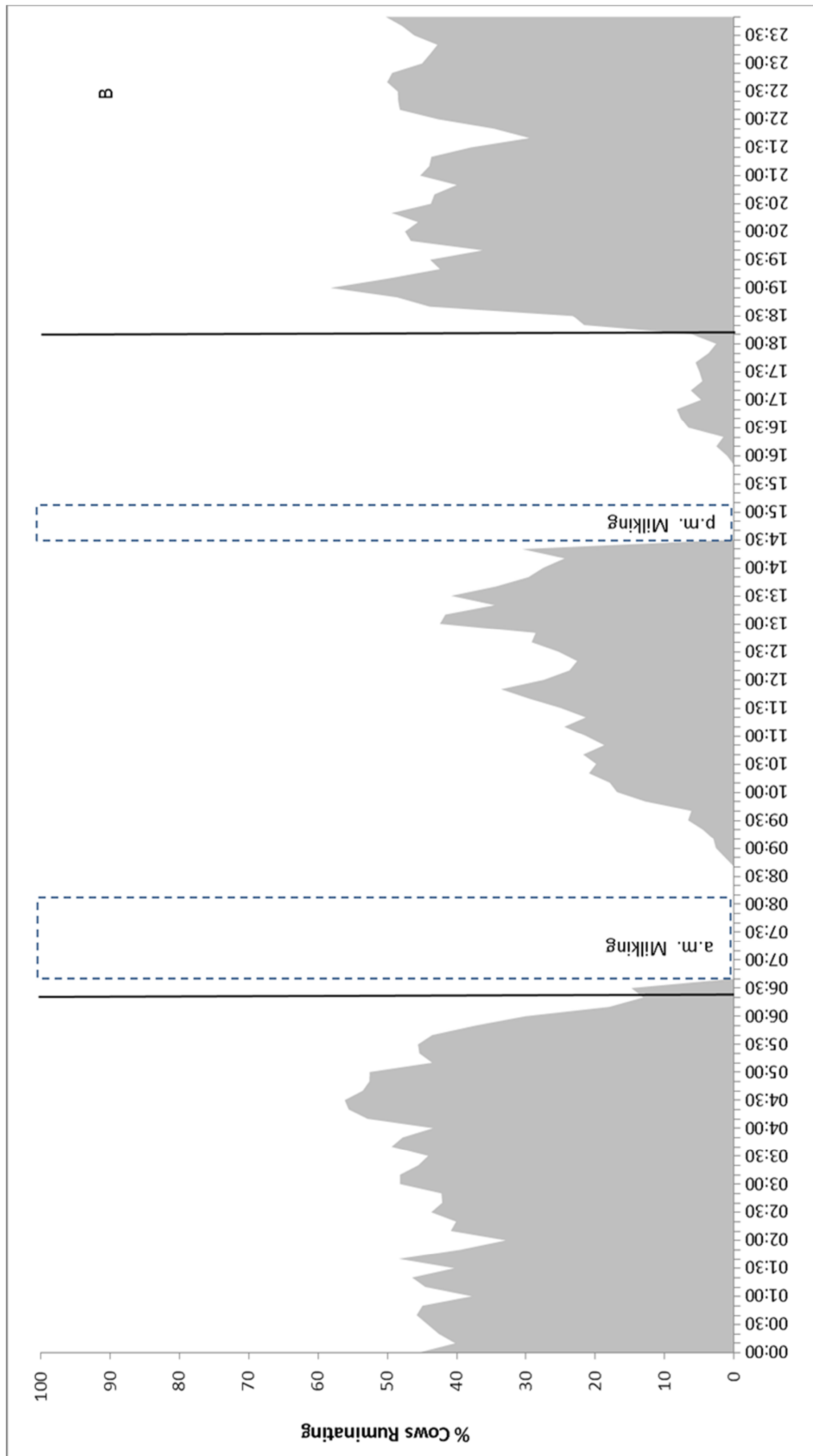
Diurnal concentrations of humoral factors are presented in (Figure 5.2 a-i). Results indicate that the main changes in concentration occurred during or after periods of grazing. Ghrelin decreased soon after grazing commenced in the a.m., but increased ($P < 0.001$) during the p.m. grazing bout until sunset, thereafter declining to its lowest measured concentration at 2000 h. Insulin concentrations increased after grazing in both the a.m. and the p.m., but the level and duration was greater in the p.m. Glucose concentrations remained constant during the a.m. grazing bout, but declined linearly ($P < 0.01$) during the p.m. grazing bout, before recovering by 2200 h. NEFA concentrations were highest prior to the a.m. grazing bout (0600 h), declined during the a.m. grazing bout to a level that was maintained until 0000 h, before rising to pre-dawn peak. During both the a.m. and p.m. grazing bouts, blood GH concentrations decreased; however, the period of decline was longer during the p.m. Concentrations of leptin gradually increased during the day, reaching maximum concentration at 2200 h, and declining thereafter. GLP-1 concentrations increased during grazing activity and in particular, during TB3, declining during TB4, when grazing activity was low. Glucagon concentrations remained fairly constant throughout the day, before increasing in the evening (1800 and 2000 h). By 2200 h, glucagon had returned to concentrations similar to those recorded at other times during the day. IGF-1 plasma concentrations did not change throughout the day.

The behaviour and blood metabolite associations are as follows. During TB1, concentrations of ghrelin (observed coefficient; OC = -0.02, $R^2 = 0.22$, $P < 0.001$) and

GLP-1 (OC = -0.12, R^2 = 0.18, $P < 0.01$) increased with decreased grazing activity (i.e. negative association), while insulin (OC = 1.41, R^2 = 0.13, $P < 0.05$) and glucose (OC = 11.4, R^2 = 0.29, $P < 0.001$) decreased (i.e. positive association). During TB2, concentrations of ghrelin (OC = 0.08, R^2 = 0.31, $P < 0.001$), insulin (OC = 1.36, R^2 = 0.05, $P = 0.05$), glucose (OC = 24, R^2 = 0.15, $P < 0.01$), and NEFA (OC = 84.4, R^2 = 0.06, $P < 0.01$) decreased as the proportion of cows grazing declined (i.e. positive association), while GH (OC = -10.5, R^2 = 0.10, $P < 0.05$), glucagon (OC = -0.17, R^2 = 0.05, $P < 0.01$), and leptin (OC = -26.9, R^2 = 0.07, $P < 0.05$) increased (i.e. negative association). During TB3, concentrations of ghrelin (OC = -0.02, R^2 = 0.53, $P < 0.001$), insulin (OC = -0.44, R^2 = 0.57, $P < 0.001$), and GLP-1 (OC = -0.04, R^2 = 0.30, $P < 0.01$) increased with decreases in the proportion of cows grazing (i.e. negative association), while GH (OC = 1.88, R^2 = 0.26, $P < 0.001$), glucose (OC = 3.07, R^2 = 0.40, $P < 0.001$), and NEFA (OC = 30.1, R^2 = 0.17, $P < 0.01$) decreased (i.e. positive association). During TB4, glucose (OC = -0.73, R^2 = 0.12, $P < 0.001$), and NEFA (OC = -13.6, R^2 = 0.11, $P < 0.05$) concentrations were negatively associated with the proportion of cows grazing, while the association between circulating GLP-1 (OC = 0.01, R^2 = 0.24, $P < 0.01$) concentration and grazing behaviour was positive.

Figure 5.1 Diurnal profiles of percentage of cows (a) grazing, (b) ruminating, and (c) idling behaviour for 17 dairy cows offered 4.4 kg/DM per day of a concentrate supplementary feed in equal portions at a.m. and p.m. milking. Vertical solid lines represent sunrise (0620 h) and sunset (1810 h). Dashed lines represent a.m. and p.m. milking.


A
a.m. Milking
p.m. Milking
% Cows Grazing
0
10
20
30
40
50
60
70
80
90
100
00:00
00:30
01:00
01:30
02:00
02:30
03:00
03:30
04:00
04:30
05:00
05:30
06:00
06:30
07:00
07:30
08:00
08:30
09:00
09:30
10:00
10:30
11:00
11:30
12:00
12:30
13:00
13:30
14:00
14:30
15:00
15:30
16:00
16:30
17:00
17:30
18:00
18:30
19:00
19:30
20:00
20:30
21:00
21:30
22:00
22:30
23:00
23:30



B
% Cows Ruminating
a.m. Milking
p.m. Milking
0
10
20
30
40
50
60
70
80
90
100
00:00
00:30
01:00
01:30
02:00
02:30
03:00
03:30
04:00
04:30
05:00
05:30
06:00
06:30
07:00
07:30
08:00
08:30
09:00
09:30
10:00
10:30
11:00
11:30
12:00
12:30
13:00
13:30
14:00
14:30
15:00
15:30
16:00
16:30
17:00
17:30
18:00
18:30
19:00
19:30
20:00
20:30
21:00
21:30
22:00
22:30
23:00
23:30

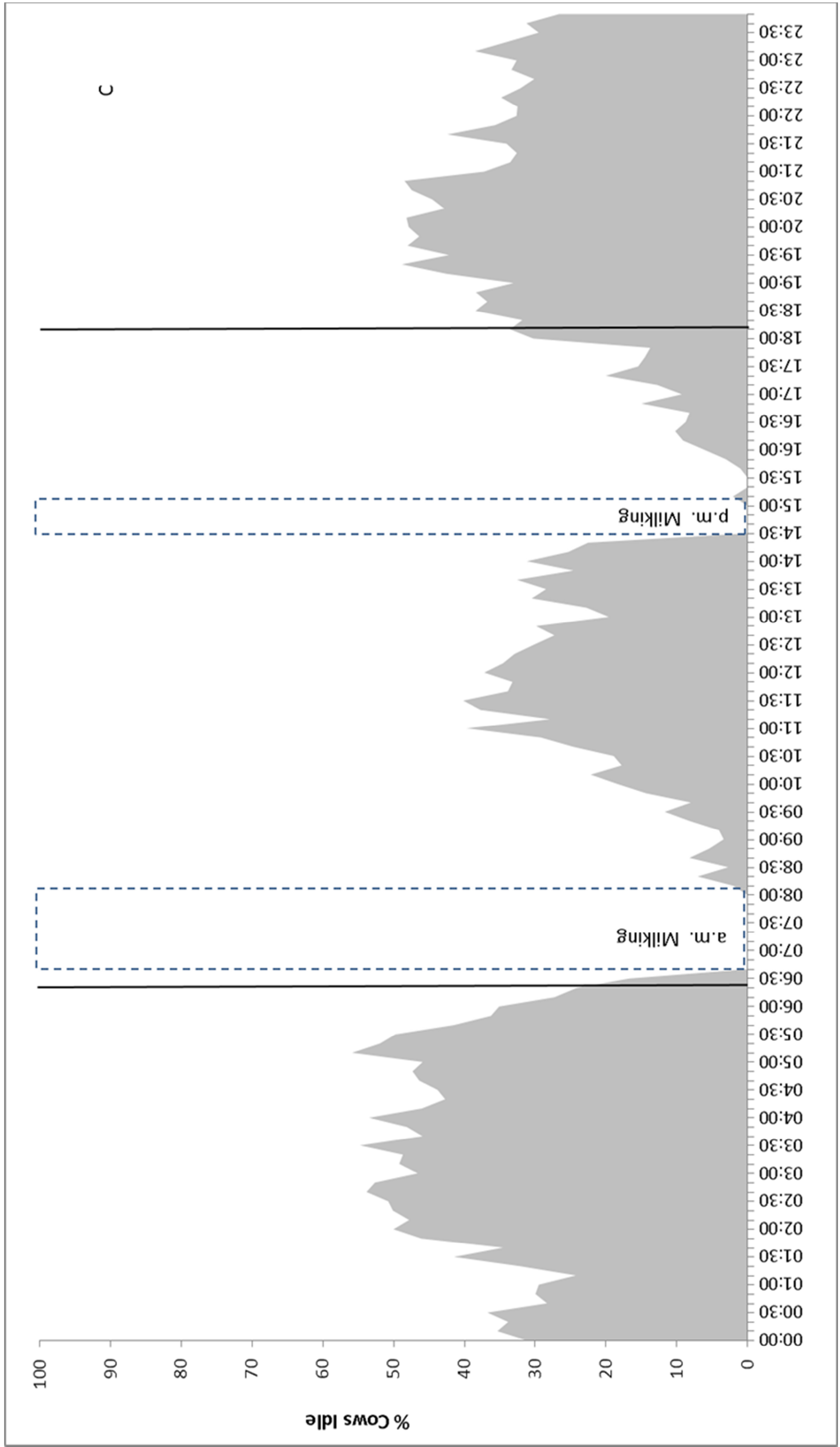
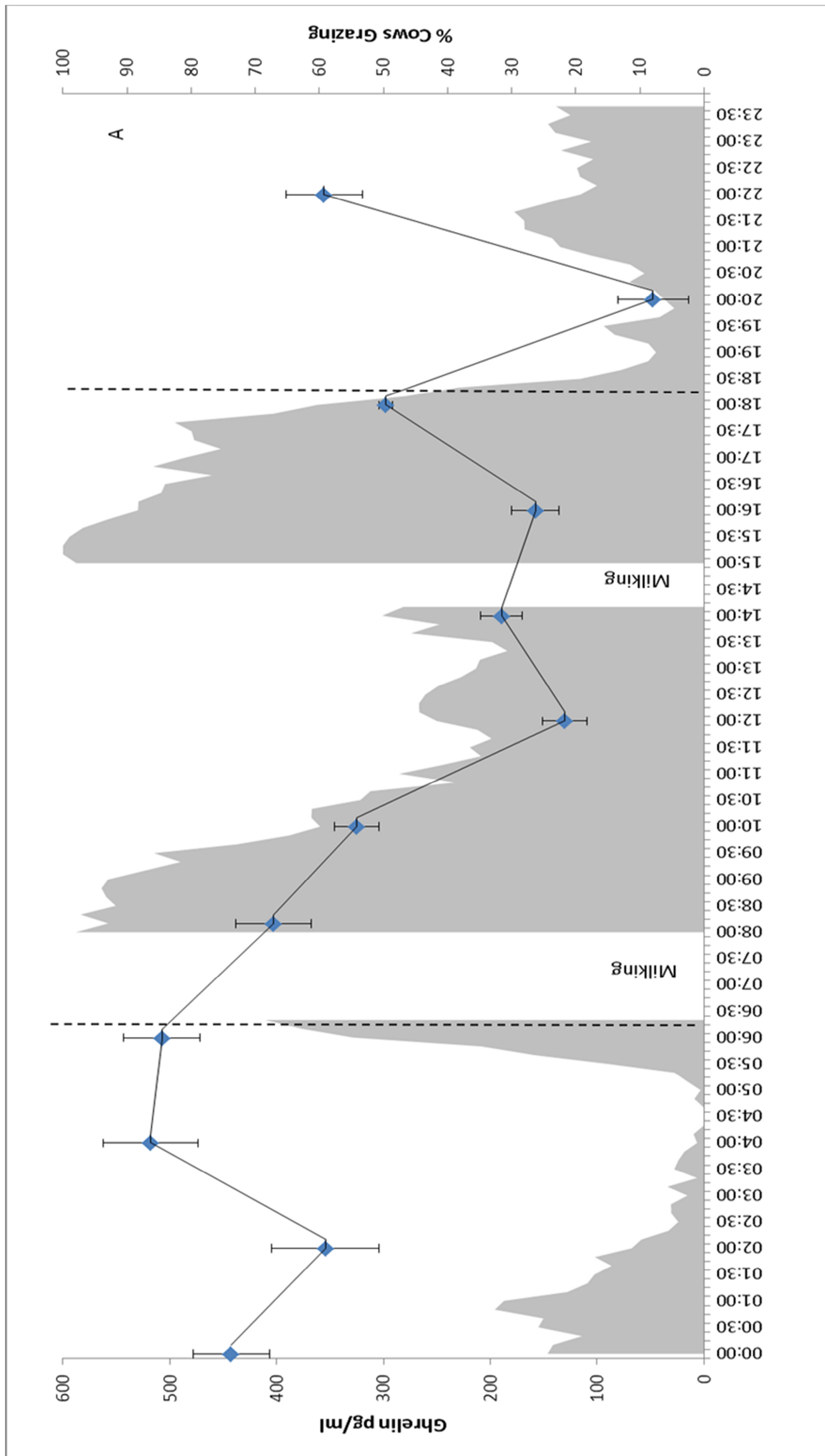
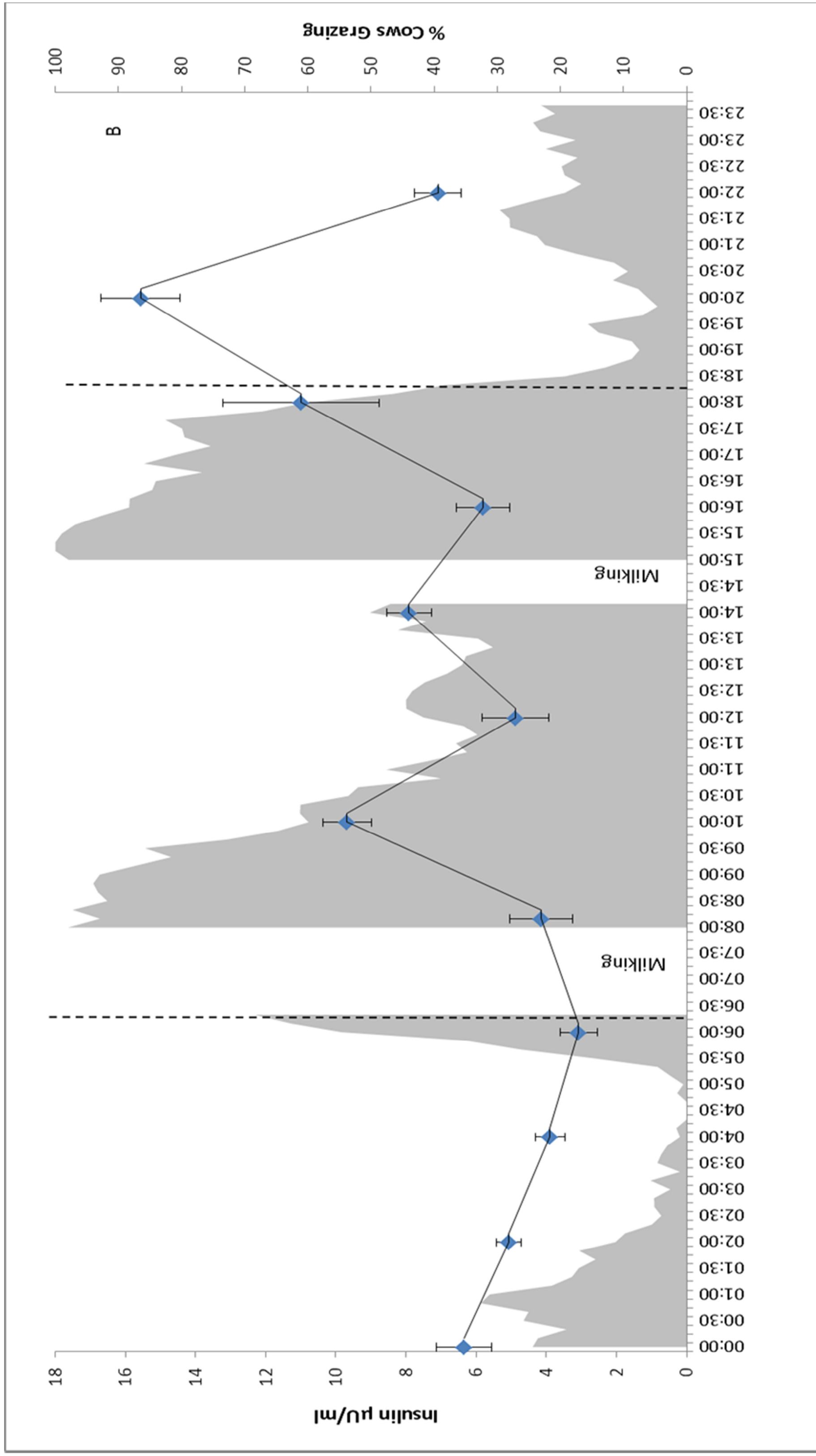
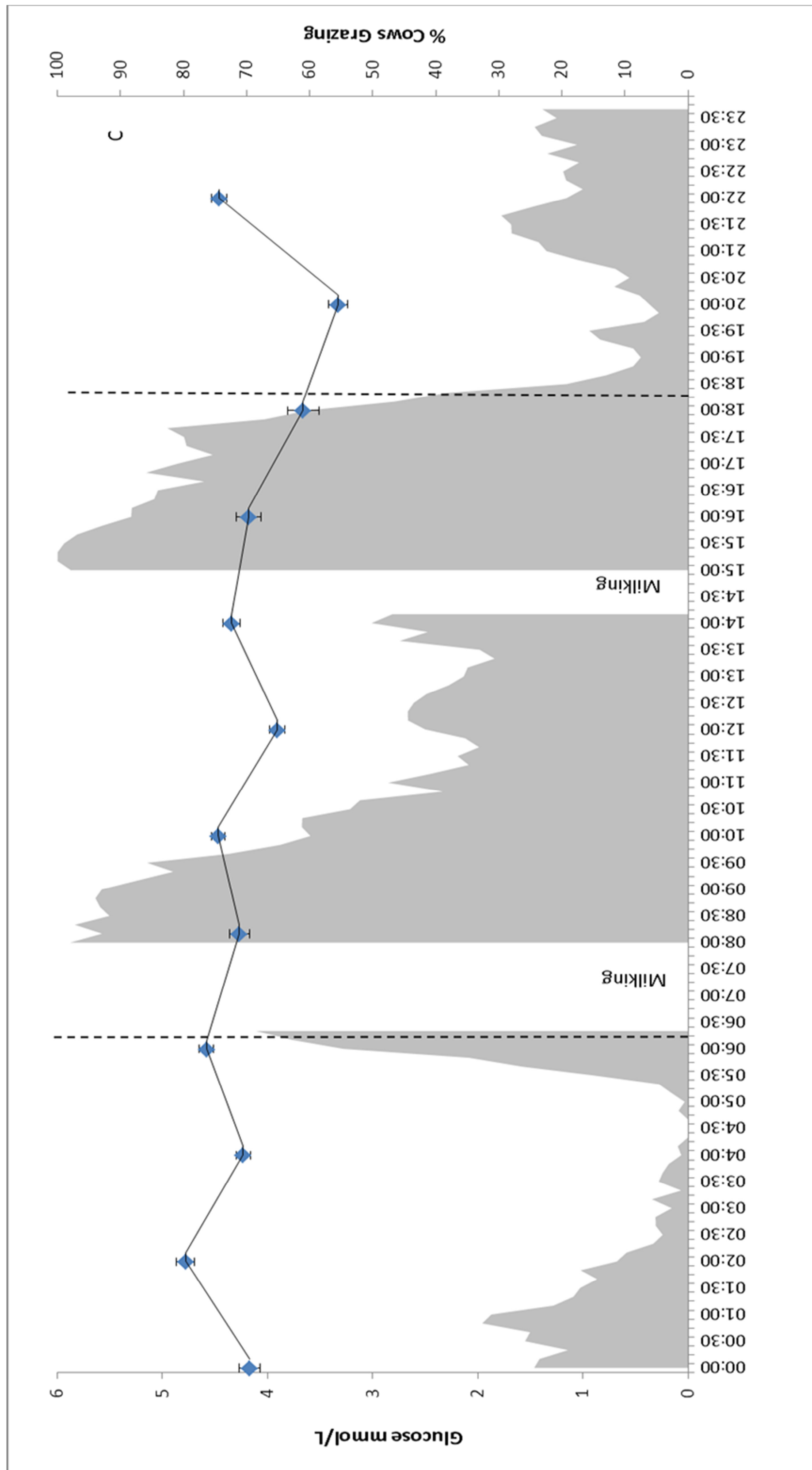

% Cows Idle
0
10
20
30
40
50
60
70
80
90
100
a.m. Milking
p.m. Milking
C
00:00
00:30
01:00
01:30
02:00
02:30
03:00
03:30
04:00
04:30
05:00
05:30
06:00
06:30
07:00
07:30
08:00
08:30
09:00
09:30
10:00
10:30
11:00
11:30
12:00
12:30
13:00
13:30
14:00
14:30
15:00
15:30
16:00
16:30
17:00
17:30
18:00
18:30
19:00
19:30
20:00
20:30
21:00
21:30
22:00
22:30
23:00
23:30

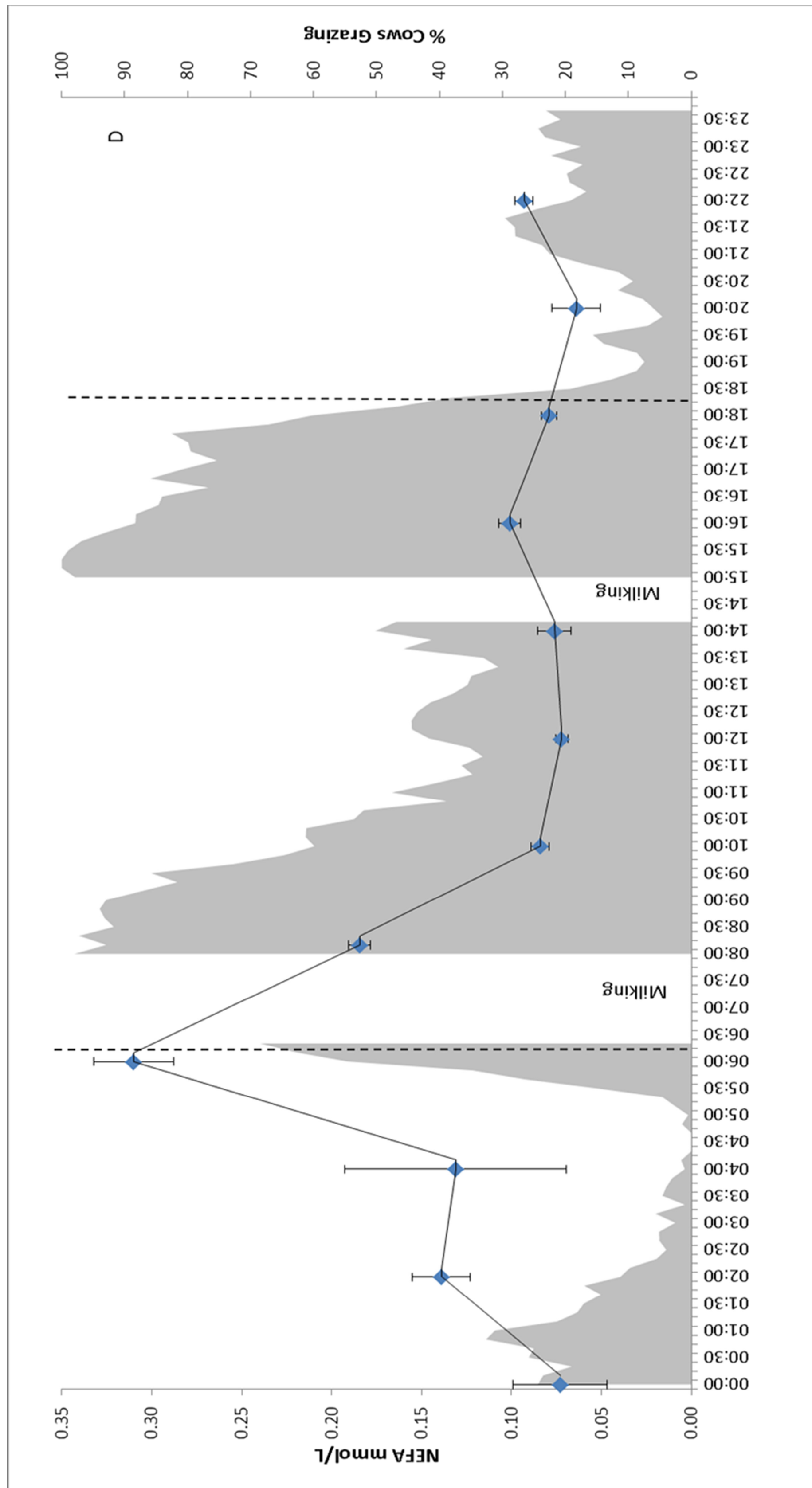
Figure 5.2. Diurnal profiles of grazing behaviour for 17 cows (proportion of cows grazing; shaded) and averaged circulating humoral factors considered to have a regulatory role in intake regulation; (a) Ghrelin, (b) Insulin, (c) Glucose, (d) NEFA, (e) Growth Hormone, (f) Leptin, (g) GLP-1, (h) Glucagon, (i) IGF-1 for 10 of the 17 cows offered 4.4 kg/day of a concentrate supplementary feed in equal portions at a.m. and p.m. milking. Vertical dashed lines represent sunrise (0620 h) and sunset (1810 h). Standard error of the mean bars is included.

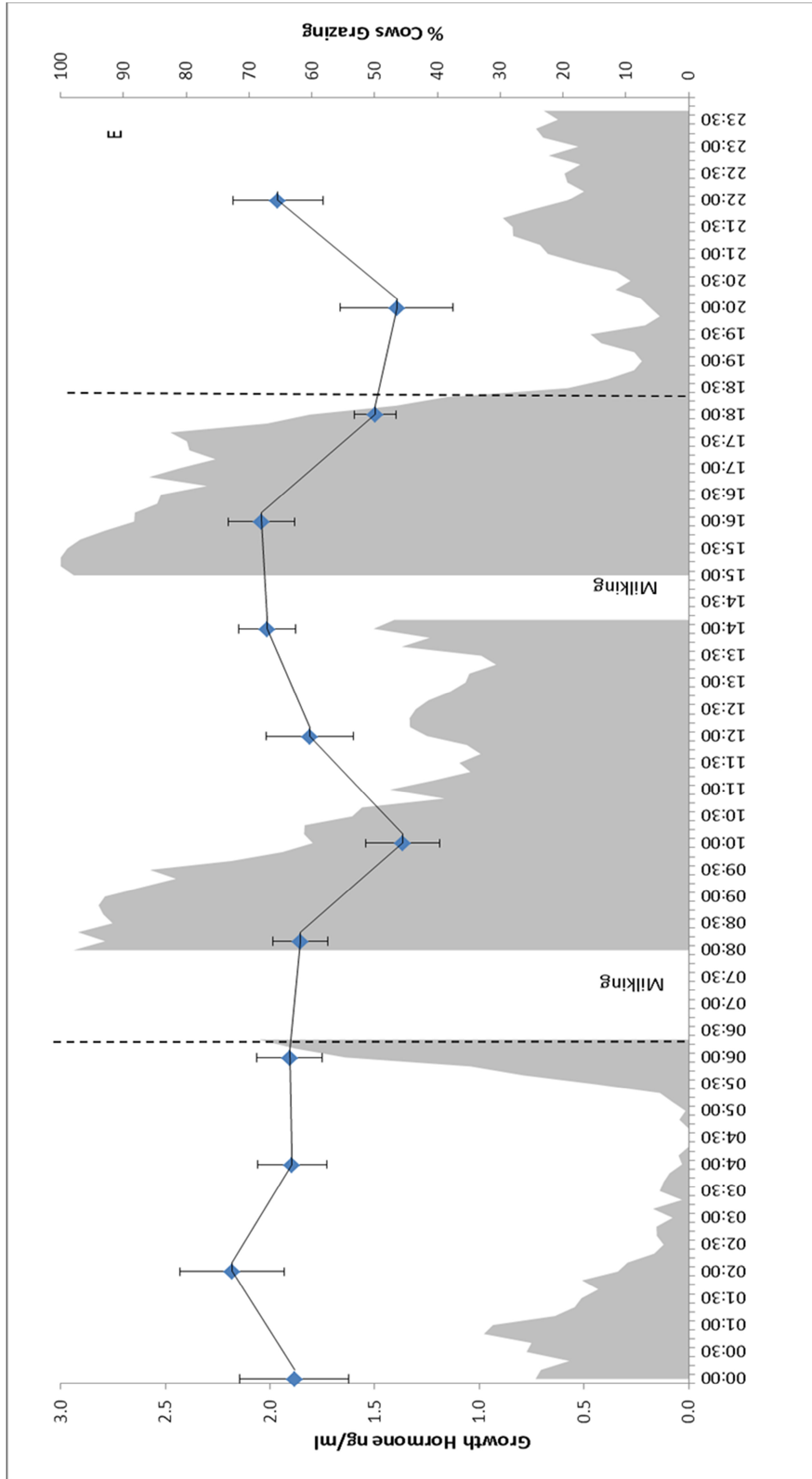


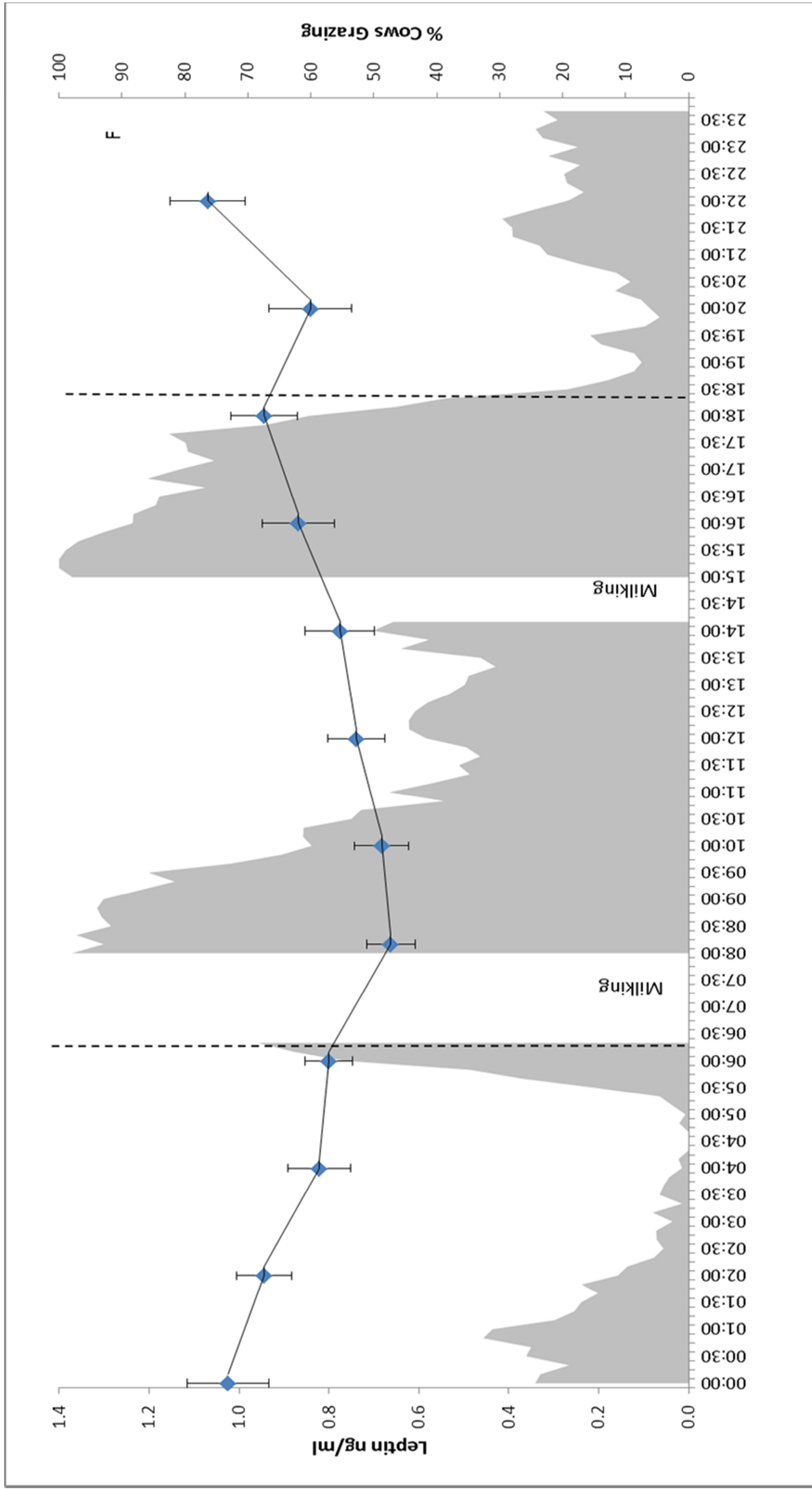
A
Ghrelin pg/ml
% Cows Grazing
Milking
Milking
0
100
200
300
400
500
600
0
10
20
30
40
50
60
70
80
90
100
00:00
00:30
01:00
01:30
02:00
02:30
03:00
03:30
04:00
04:30
05:00
05:30
06:00
06:30
07:00
07:30
08:00
08:30
09:00
09:30
10:00
10:30
11:00
11:30
12:00
12:30
13:00
13:30
14:00
14:30
15:00
15:30
16:00
16:30
17:00
17:30
18:00
18:30
19:00
19:30
20:00
20:30
21:00
21:30
22:00
22:30
23:00
23:30

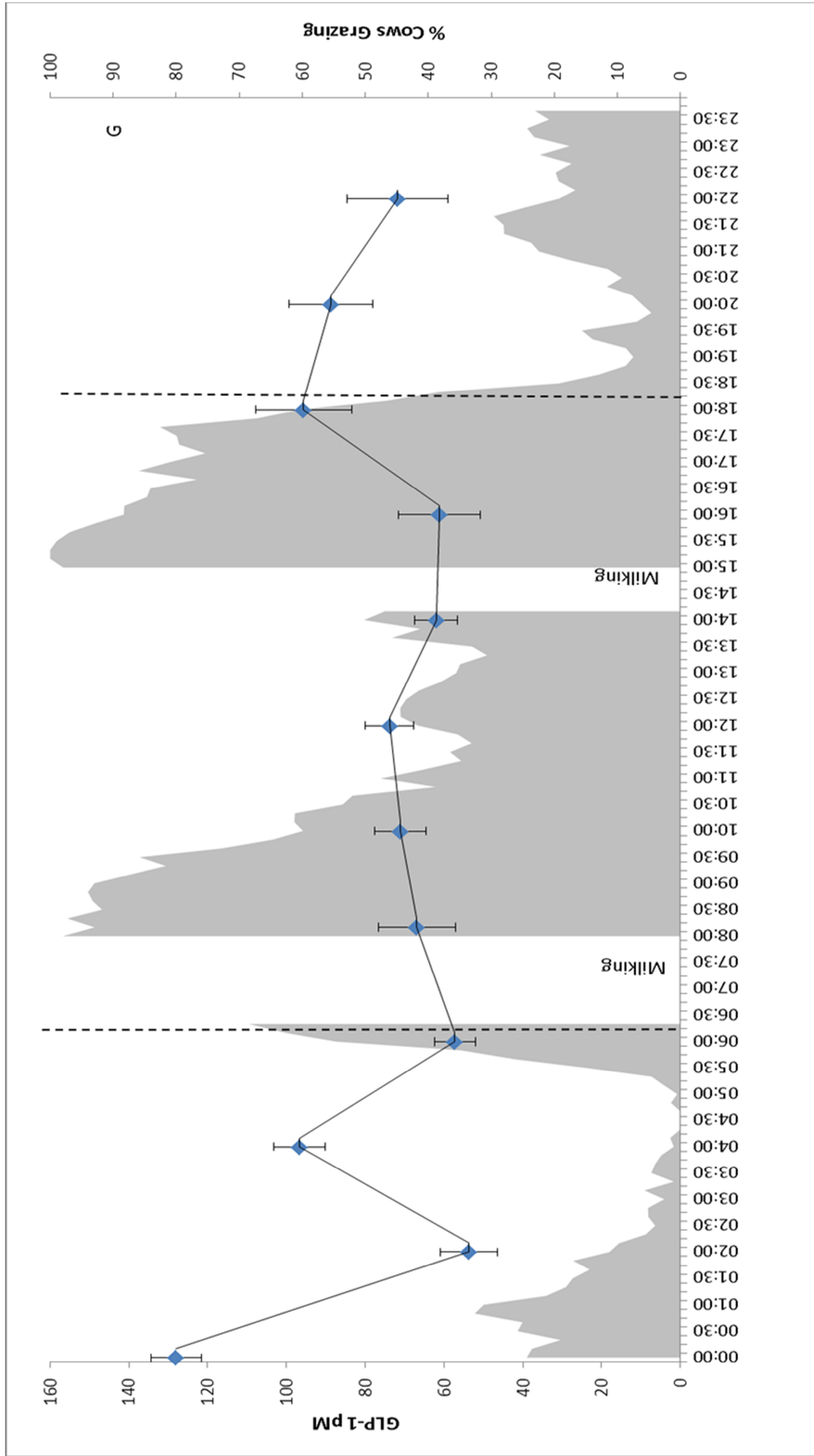

B
Milking
Milking
Insulin µU/ml
% Cows Grazing

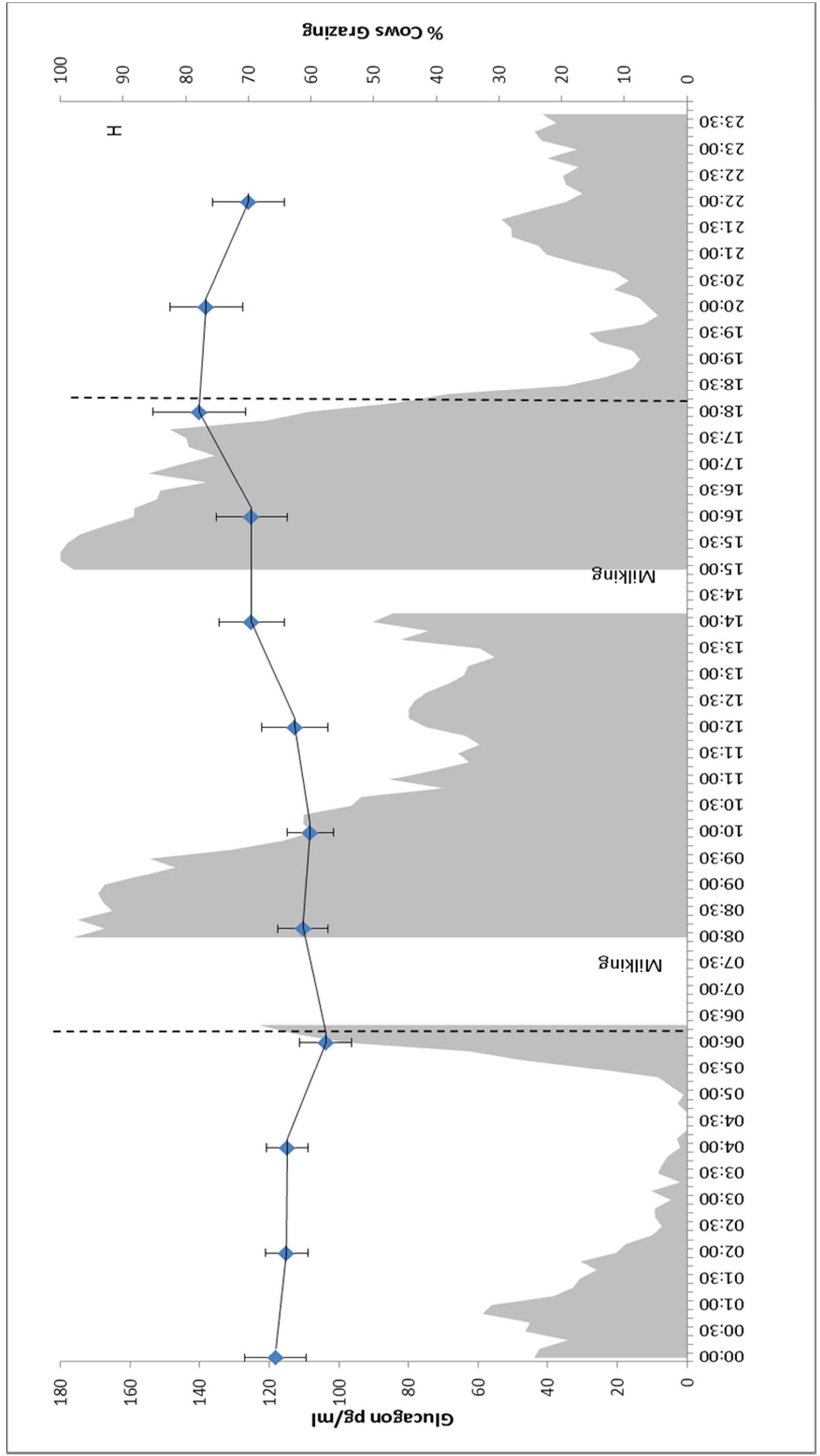

C
% Cows Grazing
Glucose mmol/L
Milking
Milking
00:00
00:30
01:00
01:30
02:00
02:30
03:00
03:30
04:00
04:30
05:00
05:30
06:00
06:30
07:00
07:30
08:00
08:30
09:00
09:30
10:00
10:30
11:00
11:30
12:00
12:30
13:00
13:30
14:00
14:30
15:00
15:30
16:00
16:30
17:00
17:30
18:00
18:30
19:00
19:30
20:00
20:30
21:00
21:30
22:00
22:30
23:00
23:30
0
1
2
3
4
5
6
0
10
20
30
40
50
60
70
80
90
100


D
NEFA mmol/L
% Cows Grazing
Milking
Milking

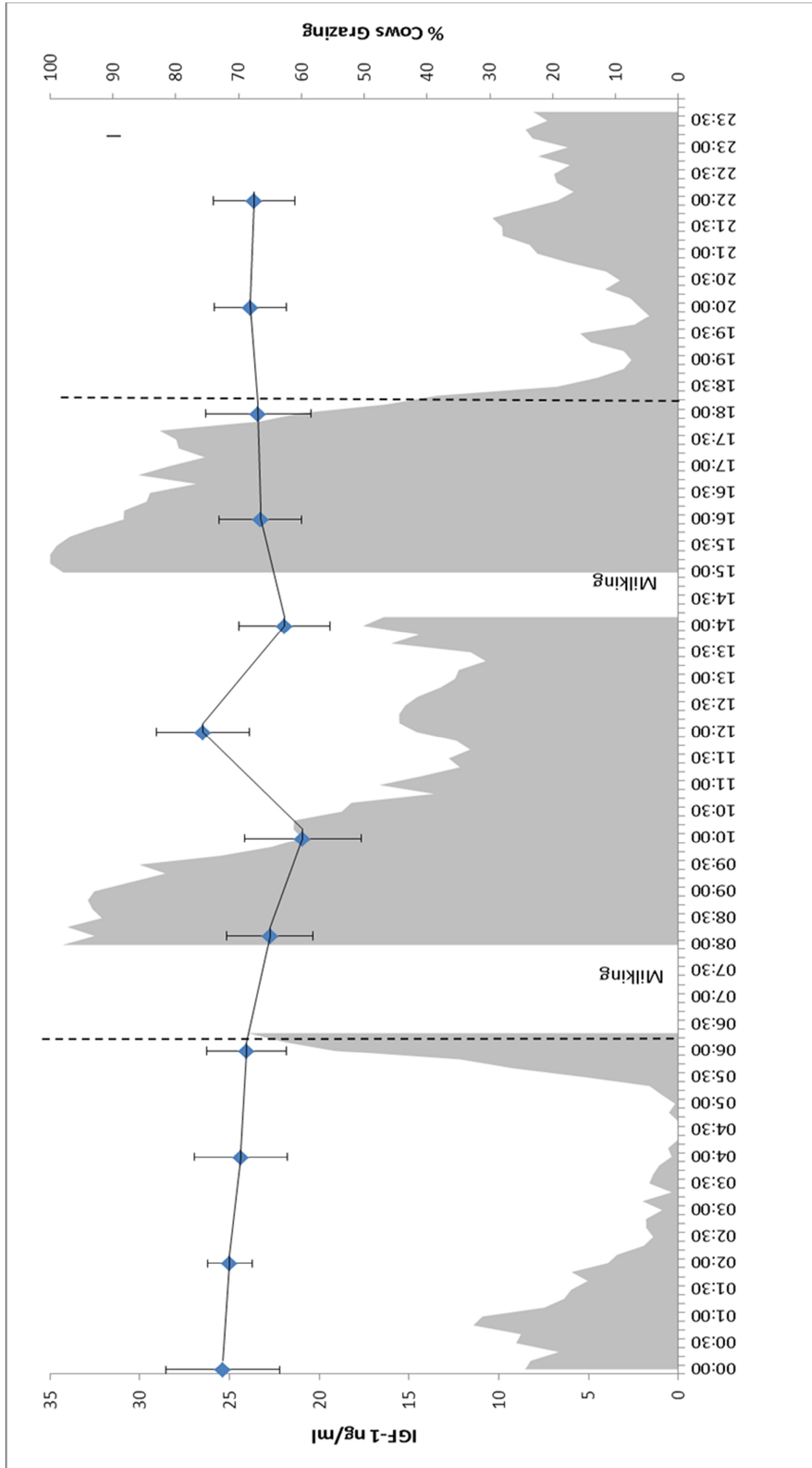

E
% Cows Grazing
100
90
80
70
60
50
40
30
20
10
0
Growth Hormone ng/ml
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Milking
Milking
00:00
00:30
01:00
01:30
02:00
02:30
03:00
03:30
04:00
04:30
05:00
05:30
06:00
06:30
07:00
07:30
08:00
08:30
09:00
09:30
10:00
10:30
11:00
11:30
12:00
12:30
13:00
13:30
14:00
14:30
15:00
15:30
16:00
16:30
17:00
17:30
18:00
18:30
19:00
19:30
20:00
20:30
21:00
21:30
22:00
22:30
23:00
23:30


F
Leptin ng/ml
% Cows Grazing
Milking
Milking


G
% Cows Grazing
GLP-1 pM
Milking
Milking
00:00
00:30
01:00
01:30
02:00
02:30
03:00
03:30
04:00
04:30
05:00
05:30
06:00
06:30
07:00
07:30
08:00
08:30
09:00
09:30
10:00
10:30
11:00
11:30
12:00
12:30
13:00
13:30
14:00
14:30
15:00
15:30
16:00
16:30
17:00
17:30
18:00
18:30
19:00
19:30
20:00
20:30
21:00
21:30
22:00
22:30
23:00
23:30



H
% Cows Grazing
Glucagon pg/ml
Milking
Milking
0
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100
120
140
160
180
00:00
00:30
01:00
01:30
02:00
02:30
03:00
03:30
04:00
04:30
05:00
05:30
06:00
06:30
07:00
07:30
08:00
08:30
09:00
09:30
10:00
10:30
11:00
11:30
12:00
12:30
13:00
13:30
14:00
14:30
15:00
15:30
16:00
16:30
17:00
17:30
18:00
18:30
19:00
19:30
20:00
20:30
21:00
21:30
22:00
22:30
23:00
23:30



IGF-1 ng/ml
% Cows Grazing
Milking
Milking
0
5
10
15
20
25
30
35
0
10
20
30
40
50
60
70
80
90
100
00:00
00:30
01:00
01:30
02:00
02:30
03:00
03:30
04:00
04:30
05:00
05:30
06:00
06:30
07:00
07:30
08:00
08:30
09:00
09:30
10:00
10:30
11:00
11:30
12:00
12:30
13:00
13:30
14:00
14:30
15:00
15:30
16:00
16:30
17:00
17:30
18:00
18:30
19:00
19:30
20:00
20:30
21:00
21:30
22:00
22:30
23:00
23:30

5.5 Discussion

The experimental objective was to investigate associations among diurnal patterns of grazing behaviour and humoral factors reported to regulate intake in non-ruminant animals. Data indicate that major periods of grazing occurred after a.m. and p.m. milking, with little grazing activity during the hours of darkness. Diurnal changes in humoral factors and associations between grazing behaviour and humoral factors are consistent with a role for these factors in regulating grazing behaviour.

The presented grazing profile is consistent with previous reports that grazing dairy cows exhibit diurnal grazing behaviour (Hafez, 1969; Krysl and Hess, 1993; Sheahan et al., 2011) with crepuscular tendencies (Gibb et al., 1998; Taweel et al., 2004; Sheahan et al., 2011). Although cows were offered equal amounts of a concentrate supplement while being milked and equal access to fresh pasture after both a.m. and p.m. milking (i.e. had the same opportunity to eat following both a.m. and p.m. milking), data indicate that grazing was more intensive in the four hours after milking in the p.m. compared with the a.m. These data are consistent with the hypothesis that different factors regulate grazing behaviour in the a.m. compared with the p.m. (Sheahan et al., 2011).

Associations between grazing behaviour during TB1, the period of lowest activity, and circulating humoral factors are similar to those reported in the literature. For example, the decrease in insulin concentration and increase in NEFA concentration during TB1 is consistent with a 'fasted' state imposed by darkness, with a reduction in circulating insulin concentration facilitating lipid secretion from adipose stores into blood (Lafontan et al., 2009). The increase in plasma ghrelin concentrations is consistent with the reported increase when ruminant (Roche et al., 2006b; Wertz-Lutz et al., 2006; Roche et al., 2007b) and monogastric species (Tschop et al., 2000; Lee et al., 2002) are in a fasted or pre-prandial state.

Cow energy status changed from a state of tissue catabolism to anabolism during TB2, as indicated by the sharp decrease in circulating NEFA concentration (Lafontan et al., 2009) shortly after grazing commenced in the a.m. The decreasing plasma ghrelin

concentration and the proportion of cows grazing during TB2 is consistent with the reported decrease in ghrelin in response to a meal in both ruminant (Sugino et al., 2002b; Wertz-Lutz et al., 2006; Roche et al., 2007b) and monogastric (Cummings et al., 2001) species. The elevated insulin concentration 2 h after the initiation of a meal (Manns and Boda, 1967; Brockman, 1978; Perboni et al., 2009) and the decrease in GH concentrations with the proportion of cows spent grazing is further evidence of a return to positive energy balance (Hove and Blom, 1973; Lafontan et al., 2009). These data indicate that, during TB2, the temporal patterns of plasma ghrelin, insulin, NEFA and GH in a grazing dairy cow are similar to those reported in monogastric species.

The increase in ghrelin concentrations during TB3 despite intensive grazing for 3 h is unique and contrary to publications stating that ghrelin decreases after meal initiation (Cummings et al., 2001; Roche et al., 2007b). Interestingly, the increase in ghrelin was also during a period of low blood NEFA concentration, and increasing blood insulin concentrations; these associations are unique in themselves, as insulin is a potent suppressor of ghrelin secretion (Flanagan et al., 2003). Roche et al. (2008c) reported an increase in ghrelin in grazing dairy cows following the insulin stimulated decline in NEFA concentration resulting from an intravenous glucose tolerance test. The increase in ghrelin, therefore, could be related to an evolutionary physiological ‘awareness’ of impending darkness, over-riding ghrelin-regulating factors associated with meal consumption to ensure that cows continue to graze until darkness.

This phenomenon has also been reported in dark phase feeders. Using mice, Murakami et al. (2002) measured an increase in plasma ghrelin concentration prior to the onset of the dark phase and a second increase during the last 3 h of the dark phase, coincident with gastric contents increasing by 50%. Bodosi et al. (2004) also reported an increase in ghrelin prior to the dark-phase in rats; however, they did not report the second peak at the end of the dark phase. This inconsistency may be due to the less frequent blood sampling regime (every 4 h) of Bodosi et al. (2004), thus missing the occurrence of the second peak. Although this area requires further investigation, current data indicate that the diurnal and possibly, crepuscular feeding behaviour of both light and dark phase feeders are associated with elevated ghrelin concentrations both prior to and towards the end of the feeding period ensuring diurnal animals maximize use of

their preferred feeding periods. This hypothesis is supported by Drazen et al. (2006), who reported a delay in the post-prandial decline in circulating ghrelin when rats were habituated to consuming their daily allowance in a 4 h period, suggesting that factors regulating feeding behaviour can be influenced by environment.

The increase in GLP-1 during TB3 may also contribute to the delay in the post-prandial decline in ghrelin concentration after a meal. Faulkner and Pollock (1991) reported that the post-prandial decline in ghrelin was suppressed in sheep infused with GLP-1. Results from the current study indicate that the suppression of the post-prandial decline in circulating ghrelin is key in facilitating the greater grazing intensity reported preceding darkness (Gibb et al., 1998; Sheahan et al., 2011) and that this feature may, itself, be regulated by humoral factors also implicated in intake regulation.

The increase in ghrelin concentrations during TB4 to levels maintained until sunrise while feeding activity is minimal is probably associated with the reported nocturnal rise in ghrelin concentration associated with sleep in monogastric species (Cummings et al., 2001; Dzaja et al., 2004). These data and the associations identified in TB4 indicate that increased concentrations of ghrelin do not necessarily reflect a physiological drive to eat and that single sample points should not be interpreted as representative of hunger. If ghrelin concentrations are to be used as an indicator of hunger, frequent blood sampling must be undertaken.

5.6 Conclusion

Diurnal profiles of humoral factors known to be associated with intake regulation in monogastric species appear to have an intake regulatory role in the grazing dairy cow. What is most interesting is the non-conventional relationship between ghrelin and grazing behaviour and ghrelin and insulin pre-sunset. The data indicate a role for ghrelin in the increased pre-sunset feeding activity in diurnal and, possibly, crepuscular animals; however, other factors may promote or temper ghrelin's role in feeding behaviour.

Chapter 6

Carbohydrate Supplements and their Effects on Pasture Dry Matter Intake, Feeding Behaviour and Humoral Factors.

6.1 Abstract

Supplementary feeds are offered to grazing dairy cows to increase dry matter (DM) and metabolizable energy (ME) intakes; however, offering feed supplements reduces pasture DM intake, a phenomenon known as substitution. The objective of the study was to investigate changes in humoral factors associated with intake regulation in monogastric species in pasture–fed dairy cows supplemented with either a starch- or non-forage fibre-based concentrate. Fifteen multiparous Friesian x Jersey cross cows were assigned to one of three treatments at calving. Measurements were undertaken in wk 8 of lactation. The treatments were: pasture only (PASTURE), pasture plus a starch-based concentrate (3.5 kg DM/cow per day; STARCH), and pasture plus a non-forage fibre-based concentrate (4.4 kg DM/cow per day; FIBRE). Pelleted concentrates were fed at an isoenergetic rate in two equal portions at a.m. and p.m. milking. Measurements were undertaken to investigate differences in pasture DM intake, feeding behaviour and profiles of humoral factors for 4 h after a.m. and p.m. milking, the periods of intensive feeding in grazing cows. Supplementing cows with STARCH concentrate reduced pasture DM intake to a greater extent than the FIBRE concentrate, although time spent eating did not differ between treatments. The humoral response to feeding differed between the a.m. and p.m. feeding events. Humoral factors associated with a pre-prandial or fasted state were elevated pre-feeding in the a.m. and declined following feeding, while satiety factors increased. In comparison, the humoral response to feeding in the p.m. differed, with responses to feeding delayed for most factors. Plasma ghrelin increased during the p.m. feeding event, despite the consumption of feed and the positive energy state remaining from the previous a.m. feeding, indicating that environmental factors (e.g. sunset) supersede physiological cues in regulating feeding behaviour. The greater reduction in pasture DM intake for the STARCH treatment in

the p.m. may be related to the level of hunger and/or satiety before the feeding event and not solely to the consumption of supplement. Data indicate that neuroendocrine factors may be responsible for the substitution of pasture for supplementary feeds.

6.2 Introduction

Low DMI is a major limitation to productivity in pasture–based systems (Kolver and Muller, 1998). Supplementary feeds are offered to grazing cows to increase DM and ME intakes; however, offering feed supplements reduces pasture DMI (Stockdale, 2000b; Bargo et al., 2003; Sheahan et al., 2011). This is known as substitution, with the pasture refused relative to supplement fed referred to as substitution rate (**SR**); SR is reflected in a reduction in grazing time (McGilloway and Mayne, 1996). Bargo et al. (2003) and Sheahan et al. (2011) reported a 12 min decrease in grazing time for every 1 kg DM concentrate supplement consumed.

However, SR is not fixed; Stockdale (2000b) reported that SR was 8-10% greater with forage supplements compared with concentrate feeds, while Stakelum and Dillon (1988) and Meijs (1986) reported greater pasture DMI when cows were supplemented with a non-forage fibre (**NFF**)-based supplement compared with an equivalent amount of energy from a starch-based supplement. These studies indicate an effect of feed composition on SR and, in particular, an effect on intake regulation.

In evaluating the primary neuroendocrine factors implicated in intake regulation in monogastric species (Arora and Anubhuti, 2006), Roche et al. (2008a) reported that these factors were also likely regulatory factors in ruminant DMI. Consistent with this, Roche et al. (2007b) reported a linear decline in plasma ghrelin 2 h post-supplement feeding in grazing cows, providing for the first time, a neuroendocrine basis for SR in grazing cows. Gibb et al. (1998), Taweel et al. (2004) and Sheahan et al. (2011; 2013a) reported that the major grazing bouts occurred immediately post-sunrise and pre-sunset and that the grazing bout pre-sunset was characterised as most intensive. However, grazing behaviour data indicate that different factors potentially regulate DMI during the post-sunrise and pre-sunset grazing bouts. Consistent with this, Sheahan et al. (2013a) reported distinct differences in the temporal profile of humoral factors implicated in intake regulation during the post-sunrise and pre-sunset grazing bouts.

Although, Sheahan et al. (2013a) identified candidate metabolites and hormones that could plausibly have an intake regulatory role in dairy cows, the blood sampling was not sufficiently frequent (every 4 h) to determine associations with certainty.

Accordingly, the objective of this study was to determine if changes in feeding behaviour coincided with changes in humoral factors using an intensive blood-sampling regime that coincided with the major feeding bouts post-sunrise and pre-sunset. In addition, the effects of supplement type on feeding behaviour, pasture DMI and the profile of humoral factors were investigated.

6.3 Materials and Methods

This experiment was conducted at Lye Farm (DairyNZ, Hamilton, New Zealand) on 24-26 August 2010, and all procedures were approved by the Ruakura Animal Ethics Committee, Hamilton, New Zealand.

6.3.1 Experimental Design

The fifteen multiparous Friesian x Jersey cross dairy cows used for this study were part of a larger experiment (Higgs et al., 2013), and had been assigned to one of three treatments at calving. Treatments were balanced for milk production (mean of the first 100 DIM from the previous lactation for multiparous cows; 17.7 ± 0.7 kg of milk/cow per day; mean ± SD) precalving BW (549 ± 29 kg), BCS (4.5 ± 0.3; 10-point scale; Roche et al., 2004), and age (4.5 ± 0.2 yr). Treatments were: pasture only (**PASTURE**), pasture plus a starch-based concentrate (3.5 kg DM/cow per day; **STARCH**), and pasture plus a non-forage fibre-based concentrate (4.4 kg DM/cow per day; **FIBRE**). Cows had been on treatment for 8 wk (56 DIM) with an average milk production of 23.1, 27.7, 26.2 (SED 1.34) kg/day for the PASTURE, STARCH and FIBRE groups respectively (Higgs et al., 2013), when this experiment commenced. Pelleted concentrates were formulated using the Cornell Net Carbohydrate and Protein System (CNCPS) v6.1 (Tylutki et al., 2008; Van Amburgh et al., 2010) and fed at an isoenergetic rate in two equal portions at a.m. and p.m. milking to supply sufficient ME and MP daily to support 30 kg of potential milk production (assuming 13 kg of pasture DMI; 11 MJ ME/kg DM; Higgs et al., 2013).

6.3.1.1 Feed Management

The current experiment required serial blood sampling every 10 min for 240 min. A major restriction to achieving this in grazing dairy cows is the logistics of taking frequent blood samples without disrupting normal grazing behaviour. As the majority of grazing activity occurs in the three to four hours post-milking, cows were offered their usual concentrate allocation and pasture allowance while tethered in a tie stall facility immediately post-milking. Cows had been previously trained to use the facility during the 2 wk leading up to the experimental measurement periods to ensure normal feeding behaviour was maintained. This was achieved by regularly tethering animals in the facility after a.m. and p.m. milking for up to 4 h with access to freshly cut pasture.

The sampling was conducted over 2 d, so that cows could exhibit normal grazing behaviour prior to each 240 min measurement period, with sampling on d 1 following a.m. milking and sampling on d 2 following p.m. milking. Throughout the larger experiment (Higgs et al., 2013), cows were offered supplements during a.m. and p.m. milking; however, on d 1 of sampling, cows were milked in the a.m. and offered supplements in the tie-stall facilities immediately following milking. Pasture was not offered until cows receiving supplement had consumed their respective supplement, which took less than 4 min. Blood samples were collected at 0 min (i.e. before supplements were offered) and every 10 min after pasture was offered to all 15 animals for a period of 240 min, after which cows were returned to the paddock. On d 2 cows were milked in the a.m. as normal and returned to the paddock; after the p.m. milking, the feeding and blood sampling protocol described for d 1 were repeated.

6.3.2 Pasture and Animal Measurements

6.3.2.1 Pasture and Supplement Intakes and Feeding Behaviour

Pre-weighed freshly cut pasture was offered to all cows individually once supplements had been consumed, which occurred within 4 min. After 240 min, pasture orts were weighed and recorded. The difference between offered and orts was recorded as pasture intake. Additional pasture was provided for cows if required and included in the pasture intake measurement. Representative samples of each supplement and

pasture offered were collected for DM and feed quality analysis. Samples were dried at 100°C for 24 h for DM analysis and 60°C for 72 h for quality and nutrient composition; dried samples were ground to pass through a 1.0-mm sieve (Christy Lab Mill, Suffolk, UK) and analysed by wet chemistry for the nutrients required for diet simulation in the CNCPS (Dairy One, Ithaca, USA; Table 6.1).

Feeding behaviour was visually assessed every 10 mins for 240 mins for every cow during both a.m. and p.m. measurement periods, with eating, ruminating or idle recorded. It was accepted that cows were in observed behaviour for the 10 mins between recordings (Gary et al., 1970). Total time spent on each behaviour was then calculated for each cow during both the a.m. and p.m. measurement periods, and analyzed as described in statistics section.

Table 6.1 Chemical composition of pasture samples fed during the a.m. and p.m. measurement periods and the starch- and fibre based concentrate supplement fed in equal portions at a.m. and p.m. milking.

	Pasture	Starch[1]	Fibre[2]
DM %	18.3	96	96.1
	% of DM		
CP	28.5	9.4	17.8
ADF	21.4	5.5	12.5
NDF	36.7	14.5	34.5
Lignin	2.1	2.1	3.5
NFC	28.9	71.1	37.9
Starch	0.5	59.5	21.7
Fat	5.3	3.7	7.3
Ash	9.83	2.25	4.66
IVTD 24hr % DM	92	94	78
NDFD[3] 24hr % of NDF	78	58	35
ME MJ/Kg DM[4]	14.1	14.5	11.71

[1]Starch and Fibre pelleted concentrates formulated using the Cornell Net Carbohydrate and Protein System (CNCPS) v6 (Tylutki et al., 2008; Van Amburgh et al., 2010)

[3] Neutral detergent fibre digestibility (NDFD)

[4]ME calculated from *in-vitro* true digestibility (IVTD) (IVTD x 0.172-1.707) (CSIRO, 2007)

6.3.2.2 Jugular Catheter and Blood Sampling

On the day prior to the a.m. intensive bleeding regime a catheter (14 gauge x 19.6 cm; Delmed, New Brunswick, NJ) was inserted into the jugular vein of each cow under local anesthesia. After each blood collection, catheters were flushed with 10 mL of isotonic saline containing 50 IU/mL of sodium heparin (Mutiparin, Fisons Pharmaceuticals, NSW, Australia).

Blood samples were collected into evacuated 10 mL tubes containing 15% K_3 EDTA, immediately placed on ice, and centrifuged at 1,500 × *g* for 12 min at 4ºC within 30 min of collection. Plasma samples were aliquoted in duplicate; one of these aliquots was acidified using 0.1 N HCl and treated with phenylmethylsufonyl fluoride

(PMSF) for ghrelin analysis (as per kit instructions; Millipore, USA). Both aliquots of plasma were stored at -20˚C until further analysis.

6.3.2.3 Plasma Hormone and Metabolite Assays

Plasma glucose, NEFA and BHBA were measured using a Hitachi 717 analyzer (Roche, Basel, Switzerland) at 30°C by Gribbles Ltd. (Hamilton, NZ). The intra- and inter-assay CV for both assays was < 2 %. Growth hormone (**GH**) was measured by RIA (Downing et al., 1995). Insulin was measured using a porcine insulin RIA kit (PI-12K, Millipore, Billerica, USA). Cross-reactivity with bovine insulin was 90%. Plasma glucagon was measured using a glucagon RIA kit (XL-85K, Millipore). The kit was specific for pancreatic glucagon and cross-reaction with oxynomodulin (gut glucagon) was < 0.1%. Leptin was measured using a 'multi-species' leptin RIA kit (XL-85K, Millipore). Ghrelin was measured using an active ghrelin RIA kit (GHRA-88HK; Millipore). Plasma glucagon-like peptide–1 (GLP-1) was measured using a RIA kit (GLP1T-36HK, Millipore), utilizing an antibody that recognizes all forms of GLP-1. The intra- and inter-assay CV for all RIAs was < 10%. Plasma neuropeptide Y (NPY) concentrations were determined by RIA (RK-049-03, Phoenix Pharmaceuticals Inc., Belmount, USA). As the kit is designed for human samples, a validation process was undertaken for use with bovine plasma. The manufactures protocol was followed and standard validations including, parallelism, repeatability and recovery, conducted. Two bovine plasma samples were serially diluted (1:1, 1:2, and 1:4) in triplicate, and yielded curves that were parallel to the standard curve, with slopes of -0.99 and -1.07 for the standard curve and serially diluted bovine samples, respectively. Recovery was tested by analysing two bovine samples, each with four different known exogenous NPY concentrations (Range = 80 - 640 pg/ml). Recovery of exogenous NPY was acceptable, with a range of 91-146%, and an overall average of 103%. Inter- and intra-assay variations were performed on six bovine plasma samples, over two separate assays, containing varying concentrations of NPY (mean range = 57-109 pg/ml). The assay produced an overall intra-assay CV of 7% ± 3.5 and an inter-assay CV of 3.2% ± 1.4.

6.3.3 Statistical Analysis

Pasture and total DMI, and the proportion of time on each behaviour activity were calculated for the a.m. and p.m. measurement periods. Each variable was then analyzed using ANOVA, firstly, including treatment, a.m. versus p.m., and the interaction of treatment with a.m. versus p.m. as fixed effects, and cow as a block effect; and, secondly, for a.m. and p.m. separately including only treatment as a fixed effect in the analysis.

Blood data for a.m. and p.m. were log10 transformed (if required) to account for heterogeneity of variance, and then analyzed separately. Repeated measurements were modeled through time using spline models within the linear mixed model framework described by Verbyla et al. (1999). Treatment, the linear trend of time and the interaction of treatment with the linear trend of time were included in the model as fixed effects. Cow, linear trend of time within cow, spline, the interaction of cow with spline and the interaction of treatment with spline were included as random effects. Residual maximum likelihood (**REML**) in GenStat 14.1 (VSN International, Hemel Hempstead) was used to fit models.

6.4 Results

6.4.1 Dry Matter Intake and Feeding Behaviour

Cows in the PASTURE treatment ate 1.7 and 1.9 kg more ($P < 0.05$) pasture DM during the a.m. and p.m. measurement periods, respectively, than cows in the STARCH group (Table 6.2). Pasture DMI for cows in the FIBRE group did not differ from either the PASTURE or STARCH treatments during either period, but was numerically intermediate. Cows in all treatments tended ($P = 0.08$) to eat more pasture DM in the p.m. than in the a.m. However, bite mass per min of feeding was greater in the p.m., with cows eating more pasture DM in a shorter time than in a.m. (Table 6.2),

Substitution rates (kg less pasture DMI relative to the control/kg of concentrate consumed) measured during the intensive 240 min a.m. and p.m. periods were affected by supplement type. The SR for the STARCH group was 1.0 and 1.1 in the a.m. and p.m., respectively, whereas the SR for the FIBRE group was 0.4 and 0.2, respectively.

Cows in the FIBRE treatment consumed 1.3 and 1.3 kg DM in total more ($P < 0.05$) in the a.m., and 1.8 and 1.9 kg DM more in the p.m. than cows in the PASTURE and STARCH groups, respectively. Combining a.m. and p.m., total DMI for the FIBRE treatment was 3.1 and 3.2 kg DM more than the PASTURE and STARCH treatment groups, respectively (Table 6.2).

Time spent eating during the 240 min measurement period in both the a.m. or p.m. was not affected by treatment Table 6.2). There was, however, an interaction between time of day and time spent eating; the PASTURE and STARCH groups spent more ($P < 0.05$) time eating in the a.m. than in the p.m., whereas time spent eating in the FIBRE group was not different in the a.m. and p.m. Rumination time was not affected by treatment during the a.m. measurement period; however, in the p.m., PASTURE cows ruminated for 24 and 26 min longer ($P < 0.01$) than cows in the STARCH and FIBRE treatments, respectively. Idling time was not affected by treatment during the a.m., but there was a tendency ($P = 0.06$) for the PASTURE group to spend less time idling in the p.m. than cows in the FIBRE and STARCH group, which did not differ from each other

Table 6.2 Pasture and total DMI (kg DM) and the proportion of time spent in eating, idle, and ruminating for 240 min after a.m. and p.m. milking. Cows (5/treatment) received pasture only (PASTURE), pasture plus 3.5 kg/day DM starch-based concentrate (STARCH), or pasture plus 4.4 kg/day DM fibre–based concentrate (FIBRE).

	PASTURE	STARCH	FIBRE	SED		*P*-value	
					Trt[1] (T)	Time (Ti)	T x Ti
Pasture DMI (kg)						0.08	0.80
a.m.	7.6[b]	5.9[a]	6.7[ab]	0.54	<0.05		
p.m.	8.2[b]	6.3[a]	7.8[ab]	0.76	0.08		
Combined	15.8[b]	12.2[a]	14.5[ab]	0.96	<0.01		
Total DMI (kg)						0.08	0.80
a.m.	7.6[a]	7.6[a]	8.9[b]	0.54	<0.05		
p.m.	8.2[a]	8.1[a]	10.0[b]	0.76	<0.05		
Combined	15.8[a]	15.7[a]	18.9[b]	0.96	<0.01		
Substitution Rate							
a.m.		1.0	0.4				
p.m.		1.1	0.2				
Combined		1.0	0.3				
Bite Mass (gms)/min feeding							
a.m.	43	35	42				
p.m.	52	43	49				
Min spent eating						0.02	0.23
a.m.	177	170	161	11.5	0.43		
p.m.	158	145	160	10.6	0.39		
Min spent idle						0.40	0.50
a.m.	44	61	72	15.4	0.22		
p.m.	46[a]	82[b]	70[ab]	13.9	0.06		
Min spent ruminating						0.10	0.40
a.m.	20	9	6	7.2	0.19		
p.m.	36[b]	12[a]	10[a]	7.7	<0.01		

[1] Treatment.

[a, b] superscript denotes difference between treatments.

6.4.2 Profile of Change in Humoral Factors Associated with DMI

Humoral factors are presented in Figure 6.1 a-h and Figure 6.2 a-f. There were changes in the humoral profiles associated with the consumption of feed during the 240 min a.m. and p.m. sampling periods.

In the a.m., ghrelin concentrations were maximum pre-feeding and declined through time ($P < 0.001$), after feeding commenced (Figure 6.1 a). In comparison, during the p.m. sampling, ghrelin concentrations increased while feeding for 30 min before declining (Figure 6.1 b). Concentrations of plasma insulin were lowest pre-feeding in the a.m. and increased ($P < 0.001$) during both the a.m. and p.m. sampling period (Figure 6.1 c and d). However, the post-prandial increase in insulin was delayed by 60 min in the p.m., relative to the provision of feed and reached greater concentrations, which were maintained until the end of the 240 min period.

Concentrations of plasma NEFA were greatest pre-feeding, particularly in the a.m. (Figure 6.1 e), with NEFA concentrations 80% lower pre-feeding in the p.m. (Figure 6.1 f) than in the a.m. Despite the different pre-feeding concentrations, NEFA declined ($P < 0.001$) during feeding in both periods.

Plasma glucose concentrations increased approximately 60 min after feeding commenced in the a.m., before declining approximately 180 min post-meal commencement ($P < 0.001$; Figure 6.1 g). In contrast, in the p.m., plasma glucose concentrations did not increase with feeding, but declined ($P < 0.001$) from baseline approximately 180 min post-feeding (Figure 6.1 h). Plasma BHBA concentrations increased ($P < 0.001$) with feeding during both periods (Figure 6.2 a and b); however, like insulin, there was a 60 min lag between commencement of feeding and the increase in BHBA in the p.m.

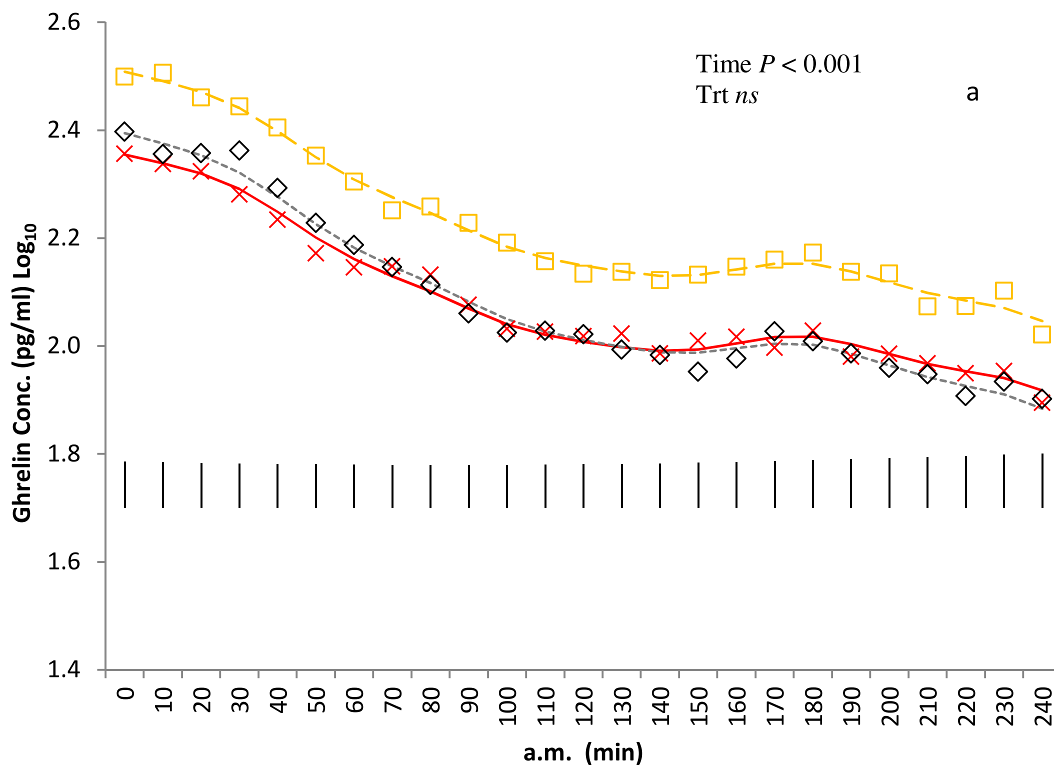
The plasma NPY profile decreased after feeding in the a.m., reaching a nadir at 120 min, before increasing ($P < 0.05$) (Figure 6.2 c). In comparison, NPY concentrations increased ($P < 0.001$) during the p.m. sampling period (Figure 6.2 d).

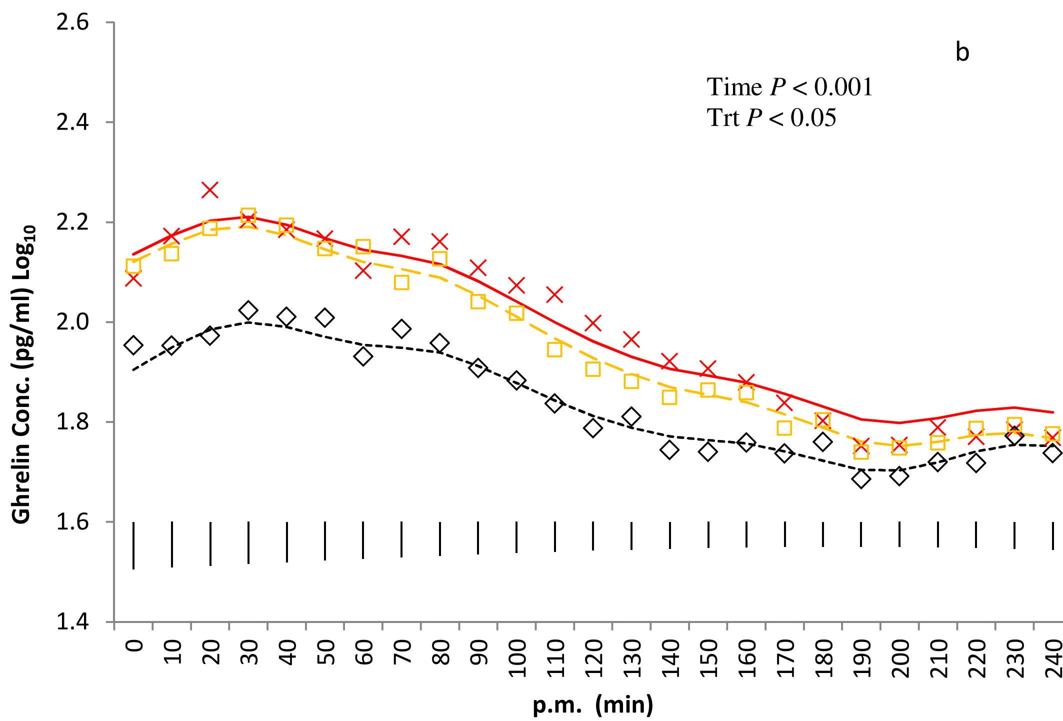
The profile of plasma glucagon was similar in the a.m. and p.m. (Figure 6.2 e and f) with glucagon decreasing ($P < 0.05$) with time. There were no effects of time or treatment for the plasma concentrations of GH, leptin or GLP-1 during either sampling period (data not presented).

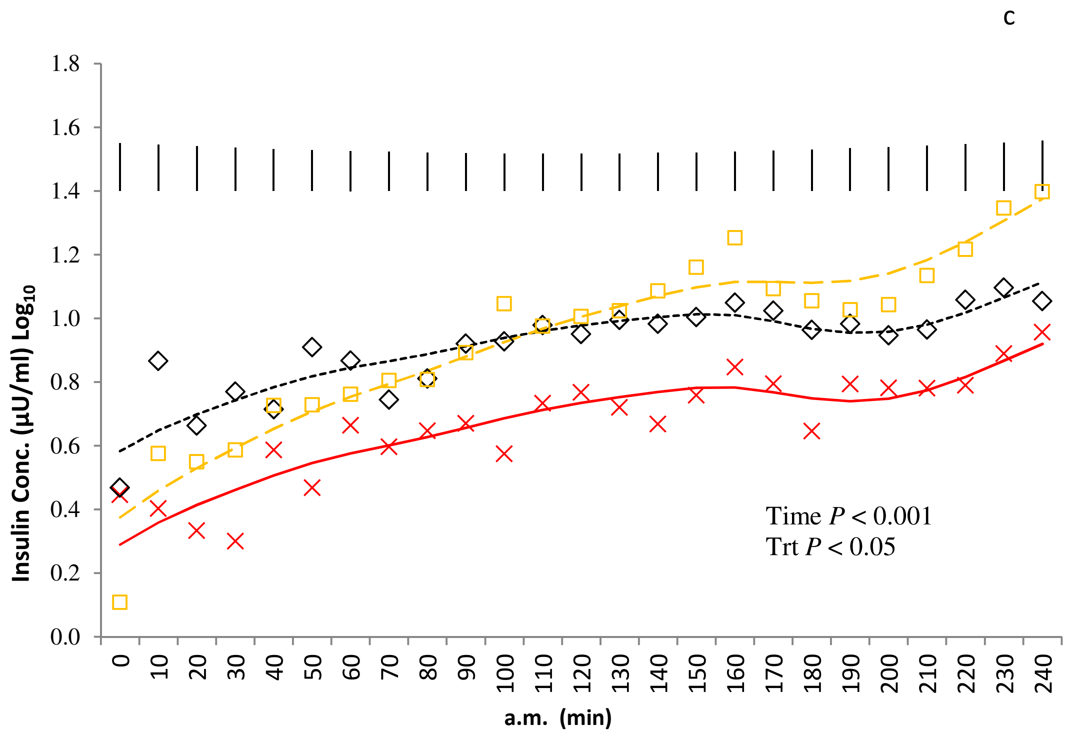
6.4.3 Supplementation Affects Humoral Intake Regulatory Factors Profiles

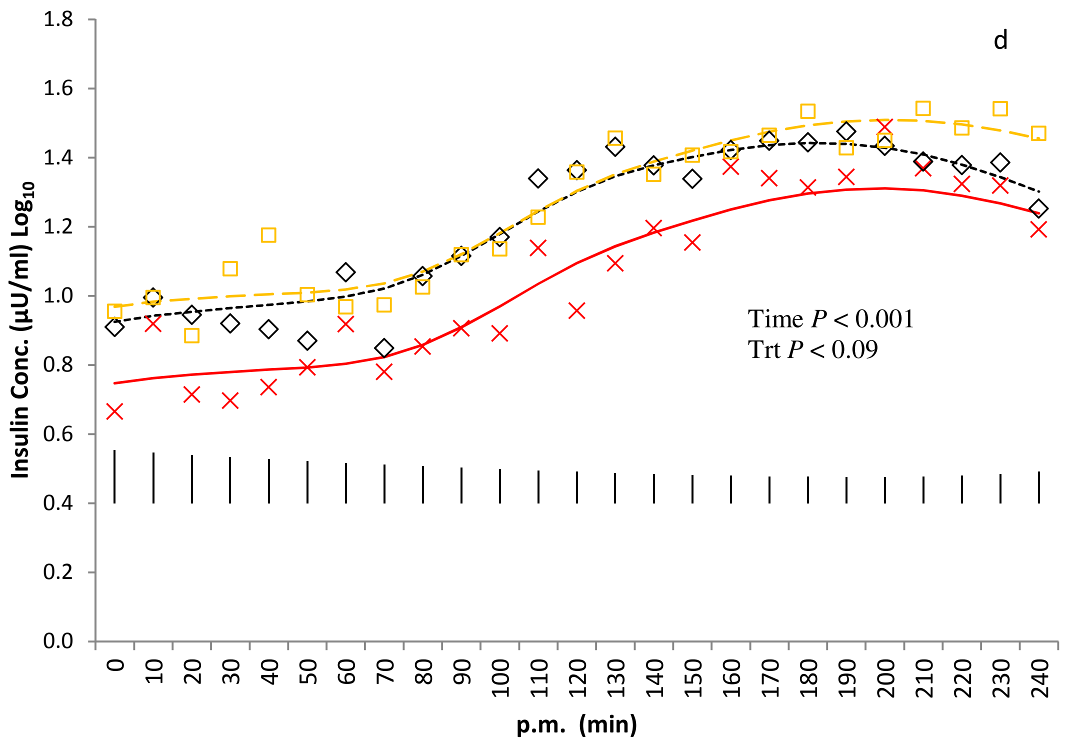
Providing cows with a concentrate supplement before access to fresh pasture affected the profiles of humoral factors measured, but with different effects for the STARCH and FIBRE treatments. The increase in plasma glucose and insulin concentrations associated with the provision of feed during the a.m. sampling period was greater in all supplemented cows ($P < 0.001$). Cows on the STARCH treatment had the lowest ($P < 0.05$) ghrelin concentration during the p.m. sampling period, with no difference between PASTURE and FIBRE treatments.

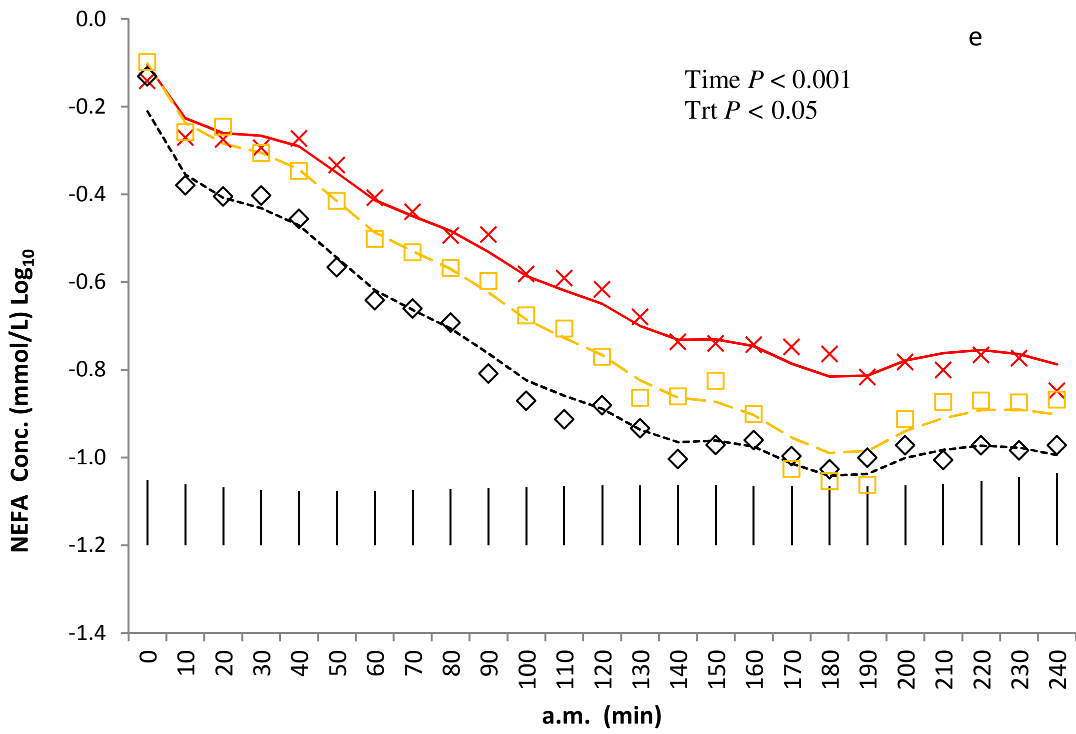
Figure 6.1 Average plasma concentrations (log10 transformed if required) wit fitted spline for 15 cows (5/treatment) offered pasture only (PASTURE ___x___), pasture plus 3.5 kg/day DM starch-based concentrate (STARCH ….◊….), or pasture plus 4.4 kg/day DM fibre–based concentrate (FIBRE ---□---) during a 240 min measurement period after a.m. and p.m. milking; (a) ghrelin a.m. (b) ghrelin p.m. (c) insulin a.m. (d) insulin p.m. (e) NEFA a.m. (f) NEFA p.m. (g) glucose a.m. (h) glucose p.m. Error bars are the standard error of the mean. Time and Treatment (Trt) effects are presented.

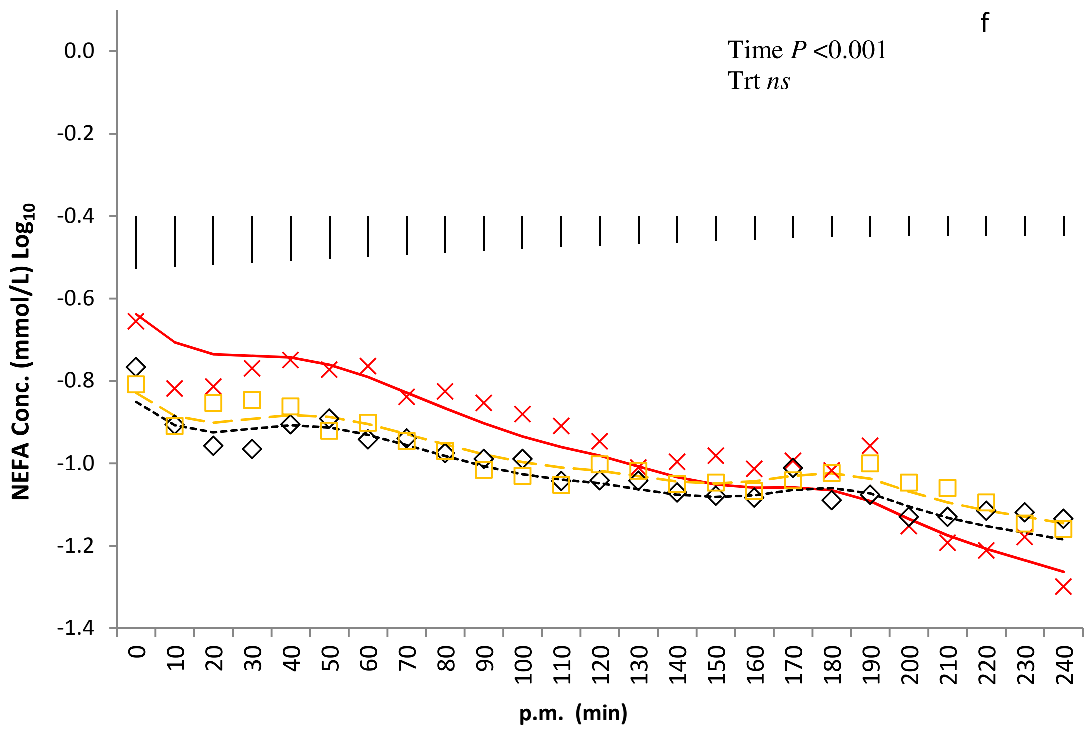

Time P < 0.001
Trt ns
a
Ghrelin Conc. (pg/ml) Log10
a.m. (min)

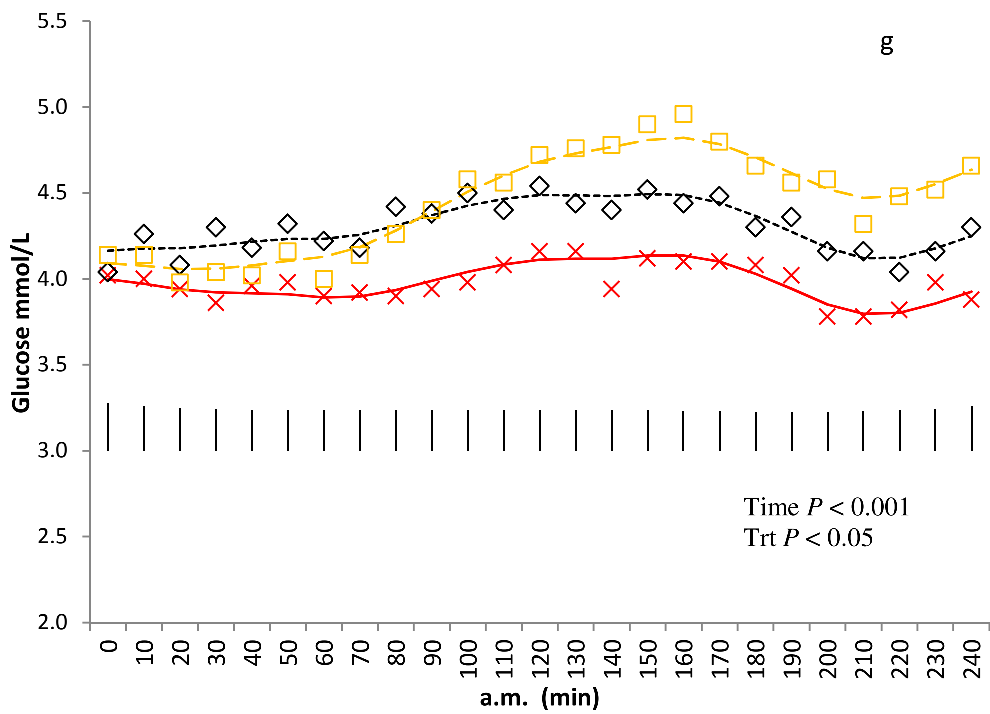

b
Time P < 0.001
Trt P < 0.05
Ghrelin Conc. (pg/ml) Log10
p.m. (min)


c
Insulin Conc. (μU/ml) Log10
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Time P < 0.001
Trt P < 0.05
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
a.m. (min)


d
Insulin Conc. (μU/ml) Log10
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Time P < 0.001
Trt P < 0.09
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
p.m. (min)


e
Time P < 0.001
Trt P < 0.05
NEFA Conc. (mmol/L) Log10
a.m. (min)


f
Time P <0.001
Trt *ns*
NEFA Conc. (mmol/L) Log10
p.m. (min)


g
Glucose mmol/L
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
a.m. (min)
Time P < 0.001
Trt P < 0.05

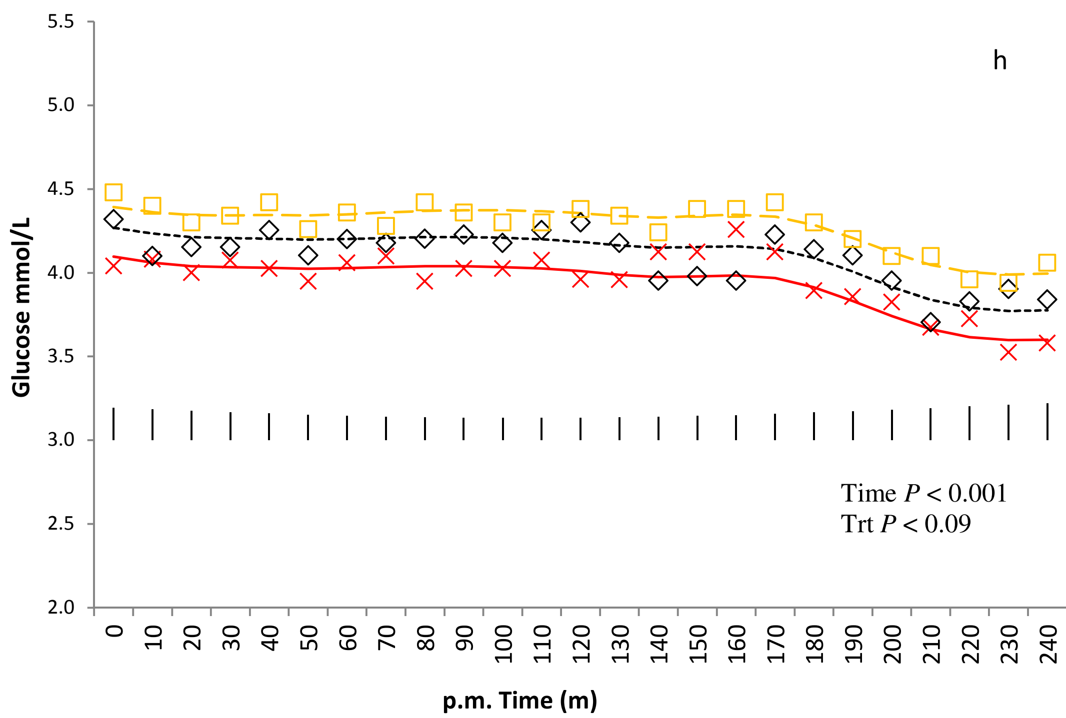
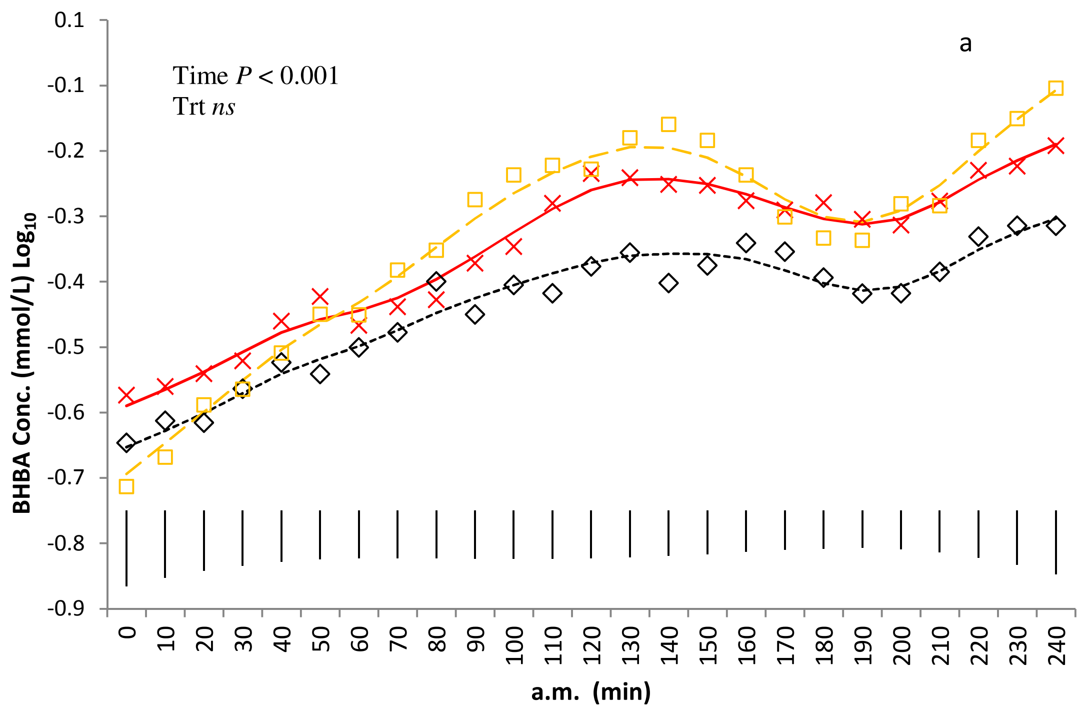
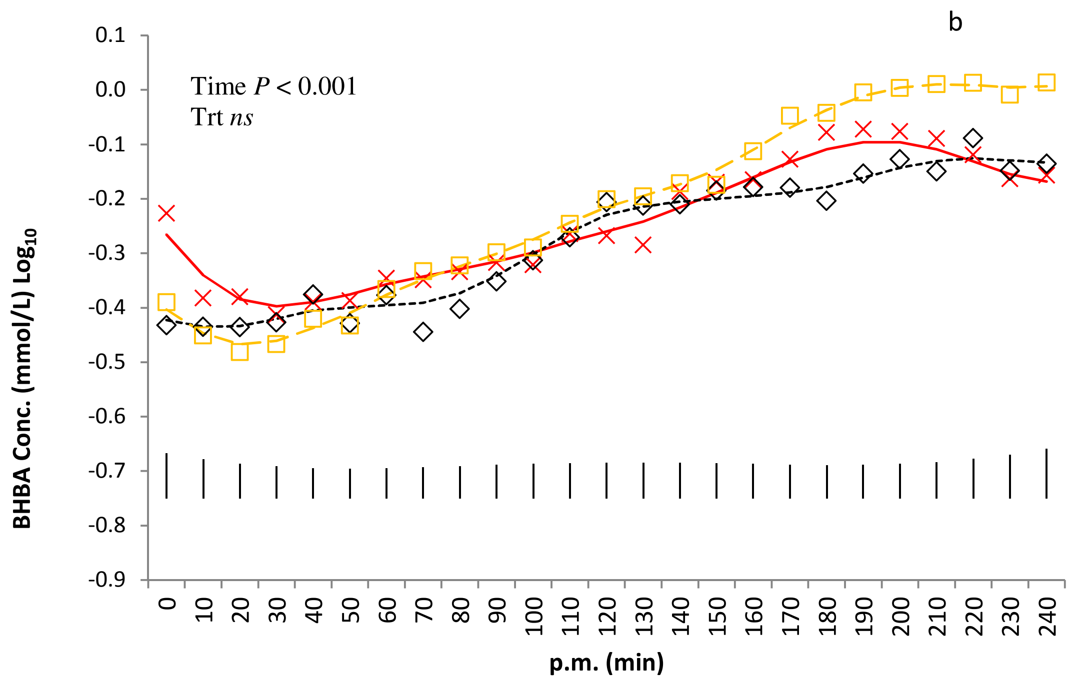
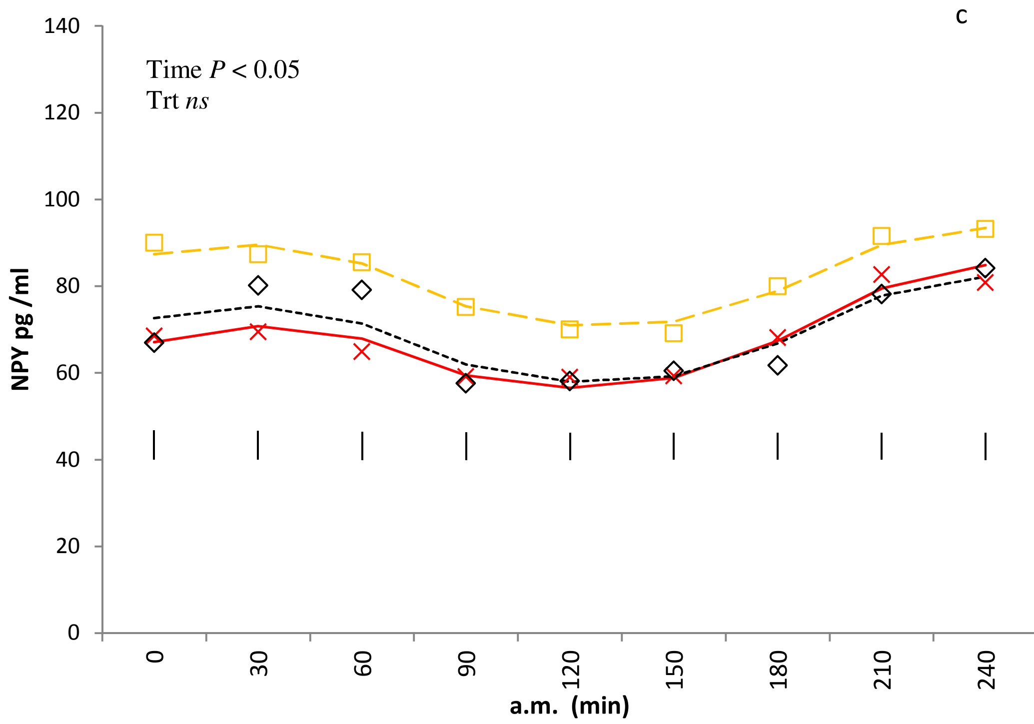
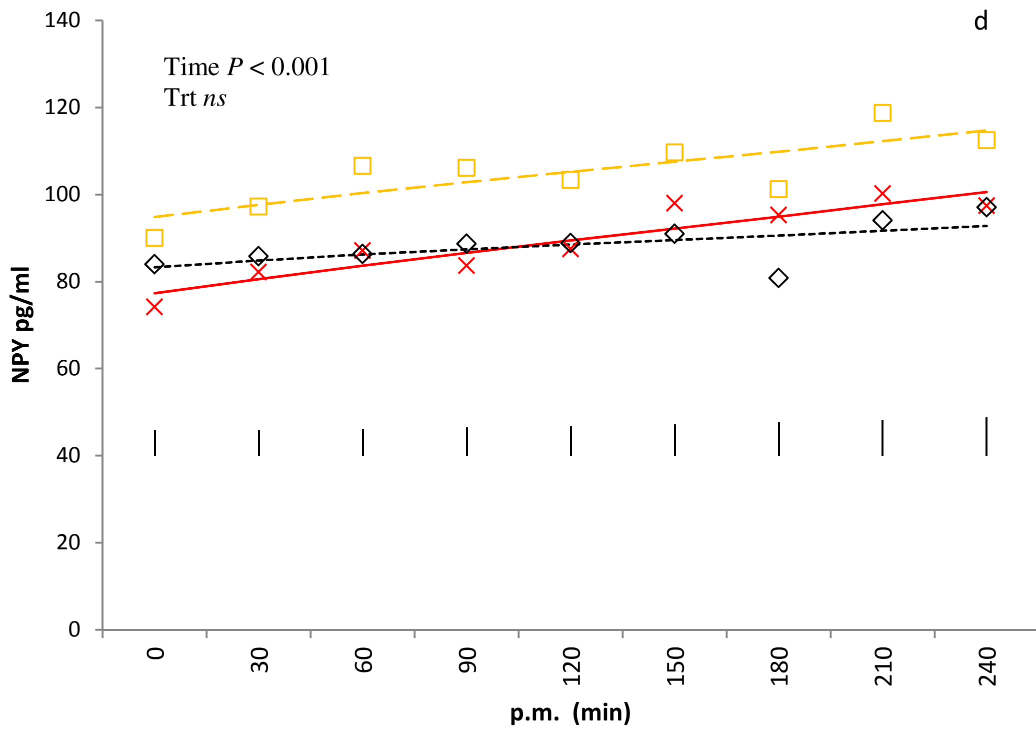

h
Glucose mmol/L
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
p.m. Time (m)
Time P < 0.001
Trt P < 0.09

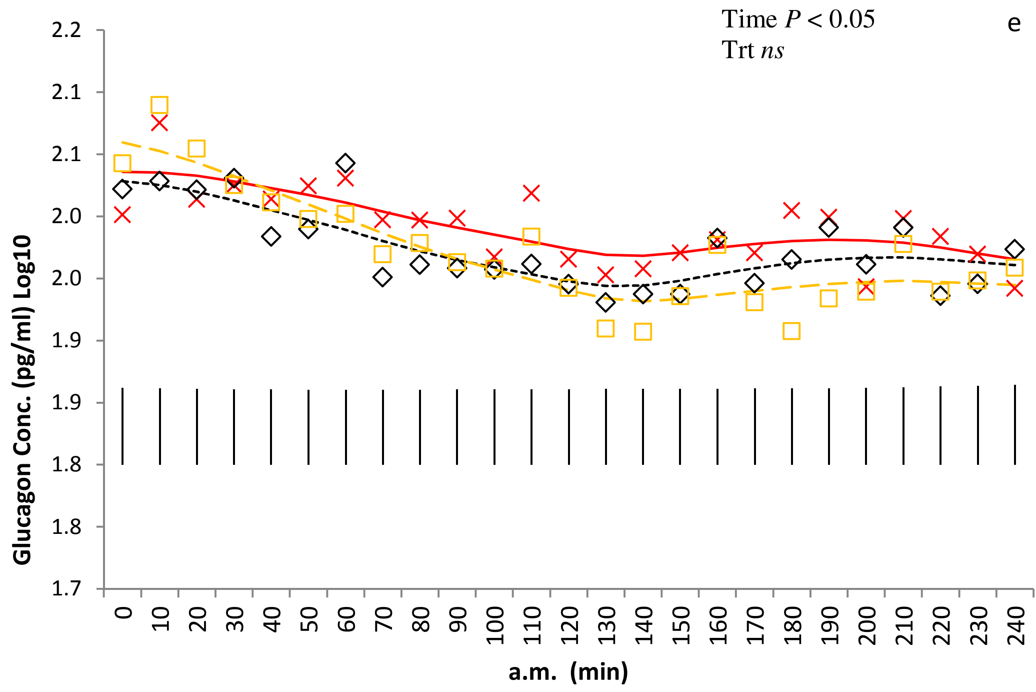
Figure 6.2 Average plasma concentrations (log_{10} transformed if required) with fitted spline for 15 cows (5/treatment) offered pasture only (PASTURE —x—), pasture plus 3.5 kg/day DM starch-based concentrate (STARCH ····◊····), or pasture plus 4.4 kg/day DM fibre–based concentrate (FIBRE ---□---)during the 240 min measurement period after a.m. and p.m. milking; (a) BHBA a.m. (b) BHBA p.m. (c) NPY a.m. (d) NPY p.m. (e) Glucagon a.m. (f) Glucagon p.m. Error bars are the standard error of the mean. Time and Treatment (Trt) effects are presented.

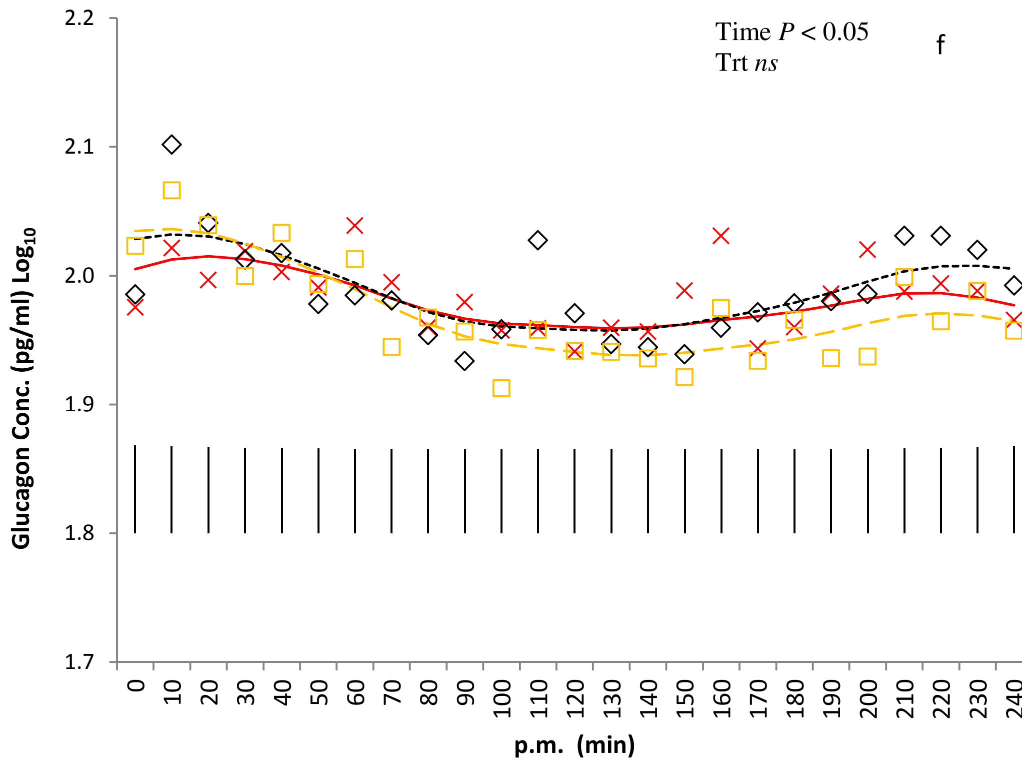

a
Time $P < 0.001$
Trt *ns*
BHBA Conc. (mmol/L) Log_{10}
a.m. (min)


b
Time $P < 0.001$
Trt *ns*
BHBA Conc. (mmol/L) Log_{10}
p.m. (min)


c
Time $P < 0.05$
Trt *ns*
140
120
100
80
60
40
20
0
NPY pg /ml
0
30
60
90
120
150
180
210
240
a.m. (min)


d
Time $P < 0.001$
Trt *ns*
140
120
100
80
60
40
20
0
NPY pg/ml
0
30
60
90
120
150
180
210
240
p.m. (min)


Time $P < 0.05$
Trt *ns*
e
Glucagon Conc. (pg/ml) Log10
2.2
2.1
2.1
2.0
2.0
1.9
1.9
1.8
1.8
1.7
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
a.m. (min)


Time $P < 0.05$
Trt *ns*
f
Glucagon Conc. (pg/ml) Log_{10}
2.2
2.1
2.0
1.9
1.8
1.7
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
p.m. (min)

6.5 Discussion

The experimental objective was to determine if changes in feeding behaviour coincided with changes in blood factors in pasture fed dairy cows supplemented with a starch- or NFF-based concentrate. The 240 min feeding periods following a.m. and p.m. milking represent the time when the majority of daily grazing occurs, as dairy cows are diurnal feeders with crepuscular tendencies (Hafez, 1969; Sheahan et al., 2011; Sheahan et al., 2013b). Therefore, although cows in this experiment were not grazing during the measurement period, we are confident that blood profiles and feeding behaviour patterns were consistent with cows in grazing conditions (Sheahan et al., 2011, Sheahan et al., 2013a). Supplementing cows with a starch-based concentrate reduced pasture DMI to a greater extent than a NFF-based concentrate, although time spent feeding did not differ between treatments. Changes in the profiles of blood factors are consistent with a role in intake regulation in dairy cows. Blood factors associated with a fasted or pre-prandial state were elevated pre-feeding in the a.m. and declined with the consumption of feed, while satiety factors increased post-feeding. The effect of supplement was also reflected in some of the blood profiles. These results support the hypothesis that blood factors associated with intake regulation in monogastric species are likely involved in intake regulation in ruminant species or that they are, at least, associated with the consumption of feed.

6.5.1 Feeding behaviour and Pasture DMI - a.m. vs p.m.

Cows spent more time eating in the a.m. than the p.m. and the pattern of feeding behaviour differed between the two measurement periods. During the a.m. feeding bout, cows ate intermittently for up to 220 min, when all cows ceased eating. In comparison, in the p.m., cows ate constantly until sunset (120 min), with feeding activity ceasing at the onset of darkness, consistent with earlier reports (Sheahan et al., 2011; Sheahan et al., 2013b). Bite mass per min of feeding was greater in the p.m., with cows eating more pasture DM in a shorter time than in a.m. This is consistent with previous research in which bite mass was reported to increase throughout the day (Gibb et al., 1998). Combined, these data indicate an effect of time of day on the intensity of

feeding behaviour; with impending darkness increasing bite mass prior to the cessation of grazing at sunset.

6.5.2 Humoral Factors - a.m. vs p.m.

The pre-feeding concentrations of humoral factors in the a.m. and p.m. were reflective of the 'energy state' and amount of feeding activity prior to the respective measurement period. Greater ghrelin and NEFA concentrations and low insulin concentrations pre-feeding in the a.m., irrespective of treatment, indicate that all cows were in a 'negative energy state' (Roche et al., 2006b; Wertz-Lutz et al., 2006; Roche et al., 2007b) consistent with the pre-prandial or fasted situation of a diurnal feeder. As feeding commenced, the decrease in plasma NEFA and ghrelin combined with an increase in glucose, insulin, and BHBA indicate a change towards a more positive energy status. These data are consistent with a response to feeding in grazing dairy cows (Sheahan et al., 2013a) and blood profiles reported for monogastric species in response to feeding (Cummings et al., 2001; Blom et al., 2005; Perboni et al., 2009).

The immediacy of the decline in NEFA (within 10 min of feed provision) and the initiation of the post-prandial decline in plasma ghrelin before the absorption of digestion products in the a.m. is consistent with the cephalic phase of gastric secretion reported in non-ruminant (Powley and Berthoud, 1985; Teff, 2000) and ruminant species (Vasilatos and Wangsness, 1980; Herath et al., 1999; Arosio et al., 2004). Wherein, the concentration of circulating factors change in anticipation of food. Thereafter, the decline in NEFA and ghrelin is most likely related to products of digestion, based on presented profiles, associated with the composition of the meal (Lee and Hossner, 2002; Erdmann et al., 2003; Blom et al., 2005; Overduin et al., 2005).

The increase in plasma ghrelin concentration with feeding in the p.m., despite the positive energy state remaining from the a.m. feeding (i.e. lower NEFA and greater insulin concentration relative to the a.m.) indicates that feeding behaviour is also regulated by non-energy state factors prior to darkness. This increase in ghrelin is not the same as the pre-feeding surges reported in monogastric species (Cummings et al., 2001) and schedule-fed sheep (Sugino et al., 2002a), as such surges occur before an anticipated meal and decline as feeding commences. Pre-prandial plasma ghrelin

concentrations in the p.m. had not returned to the pre-feeding concentrations evident in the a.m., as reported in monogastric species (Cummings et al., 2004; Blom et al., 2005), probably because of the continuous feed intake in the grazing scenario and the slow digestion and/or ruminal passage of pasture. Despite this, plasma ghrelin concentrations increased following the provision of feed in the p.m., a profile very different to that measured following feeding in the a.m. and that reported in monogastric species. This profile is consistent with the increase in ghrelin concentrations prior to sunset reported by Sheahan et al. (2013a), despite intensive grazing during this period. The increase in ghrelin in the pre-sunset feeding event may be an evolutionary adaptation in diurnal feeders to ensure feeding activity is maximized prior to sunset (Sheahan et al., 2011; Sheahan et al., 2013a), which is an environmental cue to cease feeding.

The plasma profiles indicate differences in the regulation of plasma NPY secretion between the a.m. and the p.m. periods. The plasma NPY profile during the a.m. measurement period is similar to the 'U' shaped profile reported by Sedlackova et al. (2011) in monogastric species and is consistent with the plasma ghrelin profile, possibly reflecting ghrelin's stimulatory action on NPY release (Willesen et al., 1999; Chen et al., 2004). However, the post-prandial increase in NPY in the a.m. and increase during the p.m., despite the decline in plasma ghrelin concentration, are contrary to ghrelin's stimulatory action on NPY secretion. The recorded increase is consistent with stimulation of the afferent splanchnic vagal nerve; plasma NPY concentrations are reported to increase upon electrical stimulation of splanchnic vagal nerves in the conscious calf (Allen et al., 1984). This may be similar to the increased splanchnic afferent vagal stimulation that occurs during a meal (Iggo, 1954, 1955; Date et al., 2002). Therefore, the increase in NPY in the a.m. and its continued increase during the p.m. feeding event may reflect the continuous stimulation of the afferent vagal nerve due to constant movement of digesta through the GI tract. Further research is required to investigate whether plasma NPY has a role in regulating DMI in pasture-fed dairy cows.

There was an effect of time of day on the BHBA and insulin profiles in response to feeding, with an immediate increase in both factors during the a.m., but a delayed increase in the p.m. The 60 min delay before the increase in the p.m. relative to the

a.m., may indicate a difference in the rate of VFA absorption. Although rumen or plasma VFA concentrations were not measured in the current study, inferences can be induced from other physiological data. For example, for animals in a positive energy balance, plasma BHBA represents, in part, the absorption and metabolism of butyrate through the rumen epithelium (Weigand et al., 1975; Krehbiel et al., 1992). Rumen VFA absorption is a diffusion process that is affected by the concentration gradient, rumen pH, and the saturation of the rumen epithelial cells (Bergman, 1990; Dijkstra et al., 1993). The concentration gradient would potentially be greater during the a.m. feeding event, due to low VFA concentration in the portal system, as indicated by low pre-feeding BHBA concentration. The immediate increase in plasma BHBA as feeding commences in the a.m. probably reflects an immediate uptake of rumen VFA and, consequently, stimulates a humoral response (i.e. an increase in plasma glucose and insulin and a decrease in NEFA and ghrelin). In contrast, pre-feeding plasma BHBA concentrations in the p.m. were greater than in the a.m., probably reflecting a greater VFA concentration in the portal system and, therefore, indicative of a lower concentration gradient. If this hypothesis is correct, less butyrate, and potentially, other VFA would be absorbed on commencement of feeding in the p.m. compared with the a.m., thus, delaying the humoral response to feeding. The delay in the insulin increase in the p.m., when compared with the immediate increase in the a.m., is consistent with such a delay in VFA absorption, in particular propionate (Farningham and Whyte, 1993). Therefore, profiles of plasma BHBA and insulin may indicate differences in rumen VFA absorption rates imposed by the rumen VFA concentration gradients during the a.m. and p.m. feeding periods.

6.5.3 Effect of Supplement on Feeding Behaviour and Pasture DMI

Classical theories of intake regulation imply that the capacity of the digestive tract is a limiting factor for DMI (Forbes, 2007). This may be valid for bulky, low digestibility feeds, which have long rumen residence times (Forbes, 2007), but is unlikely to be a significant contributory factor with the highly digestible pasture consumed in this study (Van Soest, 1994). Consistent with a lack of physical effect in the regulation of intake on high quality pasture, Vazquez and Smith (2000) attributed < 6% of the variation in DMI to NDF in experiments where cows were fed temperate

pastures. There is further support for this premise from the experimental results presented here. The reduction in feeding activity following the a.m. milking was well before rumen capacity could be reached and the tendency for cows to consume more DM in the p.m. and increase bite mass, despite significant feed consumption from sunrise to this point indicates that rumen fill does not play a significant role in limiting DMI when high quality pasture is fed.

The negative effect of supplementation on pasture DMI in the current study is consistent with previous reports of a reduction in pasture DMI when cows are offered supplementary feed (Stockdale, 2000; Bargo et al., 2003; Sheahan et al., 2011). However, even the most complete analysis of factors contributing to SR (Stockdale, 2000) only explains 50% of the variation in this phenomenon. Although a lower rumen pH, decreased activity or number of cellulolytic bacteria, and reduced rate of fibre digestion when starch-based concentrates are fed have been proposed as reasons for the reduced pasture DMI with concentrate feeding (Dixon and Stockdale, 1999). The limited amount of starch consumed and associated lactic acid is unlikely to create such a suboptimal environment for rumen fermentation (Russell and Hino, 1985). Furthermore, results presented here indicate a substitution effect of both the starch and NFF supplement, when the NFF supplement would not be expected to contribute to suboptimal rumen conditions.

The degree of substitution was affected by supplement type, a factor not considered by Stockdale (2000) in his review. The combined a.m. and p.m. reduction in pasture DMI was only 1.3 kg DM when a NFF-based concentrate was offered, compared with 3.6 kg DM when a starch-based concentrate was offered; this is equivalent to a 30% and 100% SR, respectively. The lower SR for the FIBRE treatment is consistent with the low SR reported by Meijs (1986) and Stakelum and Dillon (1988), when a fibre-based supplement was offered to grazing dairy cows. In the aforementioned studies, SR was calculated from daily pasture DMI; however, results from the current study indicate that the reduction in pasture DMI in response to supplementation differed with time of day for the FIBRE treatment but not the STARCH treatment (i.e. SR was less in the p.m. than in the a.m. for the FIBRE treatment). These data indicate that the carbohydrate type in the supplement has an

important role in the SR and there is an interaction between carbohydrate type and time of day on SR. An extension of this hypothesis would be that the SR is related to products of carbohydrate digestion and, possibly, subsequent effects on blood or neuroendocrine factors inducing satiation.

6.5.4 Effect of Supplement Type on Humoral Factors

The diurnal profiles of humoral factors involved in intake regulation followed a similar trajectory in all treatments; however, offering a concentrate supplement to pasture-fed cows augmented the humoral response compared with cows on pasture alone. The greater post-prandial decline in NEFA concentrations in the a.m., in particular, for the STARCH group is consistent with the provision of an energy dense supplementary feed before the cows had access to pasture that increased insulin concentrations, which facilitated the uptake of NEFA by adipose tissue (Lee and Hossner, 2002) and suppressed lipolysis (Lafontan et al., 2009). The increased glucose concentration for the supplemented groups indicate greater gluconeogenesis from exogenous, as opposed to endogenous, sources (Baird et al., 1980; Reynolds, 1992), and reflects the increased supply of glycogenic pre-cursors from the respective supplements. However, there was no difference in the plasma glucose concentrations between the supplemented groups, even though greater glucose production may have been expected from the propionate derived from the starch-based concentrate (Huntington et al., 2006). Results possibly indicate different glucogenic precursor substrates in the NFF-based concentrate; (e.g. increased oxidation of glucogenic amino acids; Reynolds, 1992) or more effective glucose uptake by peripheral tissues in the STARCH treatment (Chagas et al., 2009), although this is not reflected in circulating insulin concentrations.

The lower pre-feeding ghrelin concentration in the p.m. for the STARCH group indicates a possible prolonged satiety from the a.m. feeding event in this treatment and are consistent with the 5-fold greater SR in this treatment in the p.m. This would be consistent with the greater plasma ghrelin secretion suppression reported following a carbohydrate-rich meal compared with a protein- or fat-rich meal in monogastric species (Blom et al., 2005; Overduin et al., 2005). Sheahan et al. (2013b) referred to the

long-term effect (i.e. 16 h) of supplementary feeding on grazing behaviour. The greater SR in the p.m. for the STARCH treatment, despite an increase in total DMI when compared with the a.m., may be the result of the reduced initial ghrelin concentration affecting the 'drive to eat' and subsequent DMI, as well as the provision of additional supplement at the p.m. milking.

6.5.5 Substitution and Humoral Factors

The central thesis of the hepatic oxidation theory (**HOT**) of intake regulation proposes that the oxidation of fuels in the liver increases the energy state of hepatocytes, thereby decreasing the discharge rate of hepatic vagal afferents to the nucleus of the solitary tract, and thereby inducing satiety (Forbes, 1992; Allen et al., 2009). Although the decline in grazing time and pasture DMI with supplementation (Bargo et al., 2003) does appear to support this theory and the greater substitution rate in starch-fed cows compared with cows supplemented with a NFF-supplement is consistent with the hepatic oxidation of surplus propionate, much of the data presented do not support the HOT. For example, the elevated blood glucose concentrations pre-feeding in the afternoon and the provision of additional fermentable energy (either starch or a NFF) should result in a relatively quick decline in feeding activity, if the HOT is correct. On the contrary, however, cows continued to feed until darkness and, in fact, had the least substitution of supplement for pasture, despite the more positive cow energy status compared with the a.m. feeding event. Furthermore, the reported decrease in substitution rate from early to late lactation when a rapidly fermentable concentrate supplement was offered (Stockdale, 2000) also questions the appropriateness of HOT, as a greater substitution would be expected with advancing lactation, when a rapidly fermentable concentrate supplement is offered (Allen and Bradford, 2009) if the theory were valid. Collectively, these data question the appropriateness of the HOT for grazing dairy cows and, instead, support a role for endocrine factors that are able to communicate directly with the intake regulatory centres of the hypothalamus (i.e. ghrelin) in the regulation of the diurnal feeding pattern.

6.6 Conclusion

Offering a NFF-based concentrate to pasture-fed dairy cows resulted in lower SR than a starch-based concentrate containing equal ME, despite the greater supplement DMI. The reduction in plasma ghrelin concentration after feeding and the duration of low ghrelin concentration in starch-supplemented cows may indicate a level of satiation that potentially influences DMI at subsequent feeding events. The delay in humoral response to feeding combined with the intensity of feeding in the p.m. indicate a delay in physiological satiation, with the increase in ghrelin concentration in the p.m. ensuring feeding is maximized until sunset.

Chapter 7
General Discussion and Conclusions.

Understanding the effects of supplementation in grazing systems, on grazing behaviour and humoral profiles may help increase DMI, milk production and response to supplements. The research objectives were to use grazing behaviour to understand the negative effects of supplementation on grazing time in dairy cows, investigate the role of humoral factors known to be associated with intake regulation in monogastric species and quantify the role of theses circulating factors in pasture-fed dairy cows. This was achieved by investigating:

1. The diurnal grazing behaviour profiles in supplemented grazing dairy cows during early, mid and late lactation.
2. Whether the time that cows were supplemented (either a.m. or p.m.) altered grazing behaviour, pasture DMI and milk production.
3. Diurnal humoral profiles to determine their role in the regulation of DMI in pasture-fed dairy cows.
4. Changes in feeding/grazing behaviour in pasture-fed dairy cows with changes in humoral factors during major feeding/grazing bouts post-sunrise and pre-sunset.

7.1 Grazing Behaviour

Distinct grazing bouts were evident over a 24 hr period; grazing occurred predominantly during daylight hours, with minimal grazing during the hours of darkness. These results are consistent with Hafez, (1969), Krysl and Hess, (1993) and Gregorini et al. (2006). Presenting data as diurnal profiles of grazing behaviour (Chapter 3; Figure 3.1 and Chapter 5; Figure 5.1 a) instead of summary data of time spent grazing (Thorne et al., 2003; Linnane et al., 2004; McCarthy et al., 2007b) confirmed that sunrise and sunset were major environmental cues signalling the beginning and cessation of grazing, respectively. This effect was evident irrespective of the timing of sunrise/sunset, stage of lactation or supplementation status. These results

are consistent with Hafez (1969) and Taweel et al. (2004) who reported that cows began grazing at sunrise and ceased at sunset.

The consumption of supplementary feed reduces time spent grazing (Bargo et al., 2003; Linnane et al., 2004; McCarthy et al., 2007b). Fundamentally, however, the profile of grazing behaviour in supplemented cows followed the same pattern as unsupplemented cows (i.e. sunrise and sunset as cues to begin and cease grazing, respectively; major grazing bouts after a.m. and p.m. milking; minimal grazing during the hours of darkness, Chapter 3; Figure 3.1). The reduction in time spent grazing due to supplementation was an accumulation of reduced grazing time throughout the day and was not restricted to the period following the consumption of supplements (Chapter 3; Table 3.2). During the primary post-sunrise grazing bout, increasing the supplement level linearly reduced time spent grazing. In contrast, during the primary pre-sunset grazing bout, time spent grazing was unaffected by supplementation irrespective of level of supplementation or stage of lactation. These data are consistent with dairy cows interrupting their a.m. grazing bout long before reaching maximal rumen capacity (Taweel et al., 2004) and grazing intensity increasing prior to sunset, so that cows maximize DMI before darkness (Gibb et al., 1998). To my knowledge, the lack of effect of supplementation on grazing time during the primary grazing bout prior to sunset had not been previously reported. The differences in grazing behaviour during the major post-sunrise and pre-sunset grazing events lead to the hypothesis that different factors regulate DMI at these times.

- In the a.m., products of digestion and associated physiological factors regulate grazing behaviour.
- In the p.m., environmental cues (i.e. sunset) override physiological signals that regulate grazing behaviour in the a.m. to ensure maximal grazing occurs prior to darkness, irrespective of supplementation or energy balance status.

Based on the lack of effect of supplementation on time spent grazing prior to sunset, it was hypothesized that total DMI would be greater if cows received their supplement as a full allocation in the p.m. only, instead of equal portions at a.m. and

p.m. milking (i.e. substitution would be lower). This would allow cows supplemented in the p.m. only, to have unrestricted grazing in the a.m. (i.e. as an unsupplemented cow) and exploit the fact that supplementation does not affect grazing time in the p.m. Consistent with this hypothesis, supplementing cows in the a.m. only, reduced time spent grazing during the major post-sunrise event, while grazing pre-sunset was unaffected for the p.m. only supplemented cows (Chapter 4; Table 4.3). However, the hypothesis was rejected for two reasons:

1. Total time spent grazing was not affected by timing of supplementation, as grazing time was reduced throughout the day by supplementing at either a.m. or p.m. milking.
2. Pasture DMI was reduced in cows supplemented, to the same extent, irrespective of timing of supplementation.

These results appear to indicate an effect of nutrient consumption on time spent grazing and pasture DMI beyond the period of digestion (i.e. medium-term effect of nutrient consumption on feeding behaviour).

Supplementation affects time spent grazing and consequentially pasture DMI, as reported by Bargo et al. (2003). Yet, when cows were removed from the paddock and housed in tie-stall facilities, time spent feeding was not affected by supplementation (Chapter 6; Table 6.2). Nonetheless, pasture DMI was reduced by 1.7 and 1.9 kg/DM during the a.m. and p.m. feeding events, respectively, when cows were offered a starch-based supplement. In comparison, however, pasture DMI was not reduced to the same extent when a non-forage fibre-based supplement was consumed; this effect of carbohydrate type on substitution rate is consistent with Stakelum and Dillon (1988) and Meijs (1986).

7.2 Humoral Factors

During daylight hours, humoral profiles of factors implicated in intake regulation in monogastric species were as expected. During a fasted or pre-prandial state, as is prior to the a.m. major grazing bout (Chapter 5; Figure 5.2 and Chapter 6; Figure 6.1 and Figure 6.2), plasma ghrelin and NEFA were at their highest

concentrations and insulin and leptin were at their lowest concentrations. These data are consistent with Roche et al. (2006b), Wertz-Lutz et al. (2006) and Roche et al. (2007b), and is a humoral reflection of the minimal grazing that occurs during the hours of darkness and indicates a state of negative energy balance. After grazing/feeding commenced (Chapter 5; Figure 5.2 and Chapter 6; Figure 6.1 and Figure 6.2) the decrease in NEFA, ghrelin and growth hormone, and the subsequent increase in glucose, insulin and leptin concentrations indicate a change from a negative to a positive energy state. These results are consistent with the humoral response to food consumption in monogastric species (Licinio et al., 1998; Cummings et al., 2001; Blom et al., 2005; Perboni et al., 2009).

Ghrelin concentrations may indicate the level of hunger an animal is experiencing or the satiety imposed by a previous meal. The lower concentrations of ghrelin at the beginning of the p.m. grazing bout likely reflect a reduced level of hunger or a greater degree of satiety than pre-feeding in the a.m. (Chapter 5; Figure 5.2 a and Chapter 6; Figure 6.1 b). On this basis, the data presented indicate that the level of satiety was greater in the p.m. when cows consumed a starch-based supplement at a.m. milking than when a non-forage fibre-based supplement was consumed. This is consistent with a greater reduction in plasma ghrelin when a carbohydrate-rich diet was consumed by monogastric species, compared with a fat- or protein-rich diet (Blom et al., 2005; Overduin et al., 2005). This may explain the difference in substitution rates between starch- and non-forage fibre-based concentrates.

The profile of ghrelin during the p.m. major grazing/feeding event differed from its reported decrease in concentration after feeding, establishing a unique profile for ghrelin. Plasma ghrelin increased during the p.m. grazing /feeding event (Figure 5.2 a and Figure 6.1 b), despite intensive grazing/feeding and increased insulin concentrations, both of which are reported to suppress ghrelin concentrations (Cummings et al., 2001; Murdolo et al., 2003). This phenomenon was not previously reported in ruminant species, but a similar trend has been reported in dark-phase feeders. For example, Murakami et al. (2002) reported an increase in plasma ghrelin in mice during the last 3 hr of the dark phase, coincident with their gastric contents increasing by 50%. Due to the two hourly time points in Chapter 5 (Figure 5.2 a and b)

it could not be determined whether the increase in insulin and ghrelin concentrations during the p.m. feeding event were simultaneous. However, intensive blood sampling (Chapter 6; Figure 6.1 b) indicated plasma ghrelin increased upon feed consumption whereas, the increase in plasma insulin was delayed by 60-70 min (Chapter 6; Figure 6.1 c); this is different to the immediate insulin increase in the a.m. Whether ghrelin's increase was facilitated by the insulin delay could not be determined; however, the fact remains that cows were in a positive energy state prior to the p.m. feeding event and there was an increase in the intensity of grazing/feeding coincident with an increases in plasma ghrelin. These results led to the hypothesis that ghrelin increases in diurnal and, possibly, crepuscular species ensuring animals maximise intake prior to darkness.

Prolonged increased ghrelin concentration does not always initiate feeding. Ghrelin concentrations were greater during the hours of darkness (Chapter 5; Figure 5.2 a), which is consistent with Cummings et al. (2001) and Dzaja et al. (2004) who associated the nocturnal increase in ghrelin concentrations with sleep. Therefore, the greater ghrelin concentrations recorded at night combined with minimal grazing, even in the presence of feed, may indicate other mechanisms overriding ghrelin's intake-stimulatory effect. For example, melatonin, a hormone that increases substantially during the hours of darkness and is associated with sleep (Turek and Gillette, 2004) may have a greater intake inhibitory effect than ghrelin's stimulatory effect in diurnal species. The combined results imply that ghrelin affects hunger and satiety and, because of this, DMI, but may only effectively influence DMI during the "evolutionary preferred" grazing times (i.e. between sunrise and sunset).

The negative association between increasing insulin concentration and declining grazing behaviour (Chapter 5) is also consistent with insulin's role as a satiety factor (Woods et al., 1979; Deetz and Wangsness, 1981). However, data indicate that the role insulin plays in intake regulation in grazing dairy cows may not be directly related to cessation of feeding, a reduction in DMI, or as a measure of satiety (Schwartz et al., 2000) for the following reasons.

1. The level of insulin during the a.m. feeding period varied between treatments (i.e. greater in supplemented than unsupplemented cows), yet all cows ceased feeding irrespective of the concentration of insulin 30 min prior to the end of the measurement period.
2. The greatest insulin concentrations in the a.m. coincided with the greatest total DMI.
3. Insulin concentrations were similar in unsupplemented and supplemented cows during the p.m. feeding event despite, differences in pasture DMI.

Hepatic oxidation of nutrients has been proposed as a factor regulating DMI in ruminant species (Allen et al., 2009). The HOT states that the oxidation of fuels in the liver increases the energy state of hepatocytes, thereby decreasing the discharge rate of hepatic vagal afferents and inducing satiety (Forbes, 1992). Although the reduction in grazing time when cows are supplemented may appear to support the role of hepatic oxidation of nutrients in intake regulation, the profile of feeding in the p.m., irrespective of energy state or supplementation status do not support this theory. Prior to the p.m. primary feeding bout cows were in a positive energy state and consumed additional supplement, which will have provided additional glycogenic precursors; according to HOT, such a situation should have induced satiety. Instead, all animals, irrespective of supplementation status, increased intensity of feeding (i.e. ate more pasture DM in a shorter time interval than the a.m.) and continued to feed until the onset of darkness, when all feeding ceased. This is inconsistent with HOT. These results indicate that the hepatic oxidation of nutrients is not the primary regulator of DMI in grazing dairy cow.

7.3 Conclusions of this Thesis

By investigating grazing/feeding profiles, two main conclusions were deduced.

1. Sunrise and sunset are major environmental stimuli for the beginning and cessation of grazing, irrespective of stage of lactation, supplementation status or timing of sunset.
2. The lack of effect of supplements on time spent grazing pre-sunset, indicate that environmental signals (i.e. sunset and impending darkness) override physiological signals to ensure feeding is maximised within the preferred "evolutionary" feeding times. However, the intensity of feeding (i.e. bite mass per min feeding) is impacted by the consumption of feed and this effect is modified by feed composition.

The monitoring of humoral factors combined with grazing/feeding behaviour indicated the energy state of the animal and the degree of hunger and/or satiety prior to the onset of the major feeding bouts, and the humoral response to the consumption of feed. However, the major finding in the research undertaken was the increase in plasma ghrelin combined with the increased intensity of feeding during the primary p.m. feeding bout prior to sunset, despite animals being in a positive energy state. The increased ghrelin during the p.m. major grazing/feeding event differed from its reported decrease in concentration after feeding, thereby, establishing a unique profile for ghrelin. It was, therefore concluded that the pre-sunset increase in plasma ghrelin in diurnal species ensures animals maximise DMI prior to darkness, which is a major environmental cue to cease grazing/feeding.

Appendix A

Orexigenic and Anorexigenic Signals Regulating Intake.

Irrespective of animal species, the classification of an orexigenic (feed stimulating) or anorexigenic (feed inhibiting) hormone, neurotransmitter or other internal signal must fulfil key criteria.

1. The signal must circulate in either direct or inverse proportion to the degree of adiposity, with concentrations modified reciprocally with changes in adipose stores.
2. It must gain access to the brain and interact with the receptors and neurons known to regulate energy balance.
3. Exogenous either (centrally or peripherally) administration affects food intake or meal size.
4. Blocking (antagonists) or deletion of its endogenous activity affects food intake or meal size.
5. A reduction in food intake caused by administration of an ‘anorexigenic’ signal should not be the consequence of illness or malaise, or of some sort of incapacitation.
6. The secretion of endogenous orexigenic signals must follow a period of fasting. Similarly, the secretion of endogenous anorexigenic signals must be elicited by ingested food, with a temporal profile consistent with contributing to the normal cessation of eating.
7. Chronic infusions should alter body fat mass and the responsiveness of peripheral tissues to energy.

Important advances have been made in the characterization of hypothalamic neuronal pathways and neuropeptide transmitters, along with circulating peptides that relay signals to the brain regarding the body’s nutritional status (Stanley et al., 2005). The major sources outside of the hypothalamus are the gastrointestinal tract, adipose tissue and pancreas.

A.1 Central Intake Regulation Factors

A.1.1 Orexigenic Peptides

A.1.1.1 Neuropeptide Y

Neuropeptide Y (**NPY**) is a 36-AA peptide produced in abundance in the hypothalamus (Allen et al., 1983b) and has been identified throughout the peripheral nervous system (Gu et al., 1983) and the adrenal medulla (Allen et al., 1983a). NPY is recognised as one of the most potent orexigenic factors known (Edwards et al., 1999).

Central administration of NPY leads to a state of positive energy balance and increases fat storage, by increasing food intake, decreasing energy expenditure and increasing lipogenesis by stimulating the expression of lipogenic enzymes in white adipose tissue (Billington et al., 1991; Schwartz et al., 1992). A single intracerebroventricular (**ICV**) administration of NPY acutely stimulated feeding in rats (Clark et al., 1984), and prolonged hypothalamic administrated NPY produced hyperphagia and obesity in rats (Stanley et al., 1986). Although NPY neuronal expression is a potent orexigenic stimulus, the absence of NPY (in knockout studies) did not result in the cessation of feed intake (Erickson et al., 1996; Pedrazzini et al., 1998).

Five NPY receptors have been identified Y1-6 (Wahlestedt and Reis, 1993), but only the Y5 receptor has been implicated to mediate the feeding effects of NPY (Marsh et al., 1998; Pedrazzini et al., 1998). The Y5 receptor is expressed at high levels in the lateral hypothalamic area (**LHA**), close to the site where NPY acts most potently to stimulate feeding (Williams et al., 1989).

The level of NPY signalling is influence by nutritional status (Williams et al., 1989), as fasting and food restriction increase NPY mRNA in the ARC (Sahu and Kalra, 1993; Schwartz et al., 1996). Smith (1993) reported an increase in NPY mRNA during lactation when energy demands are high, coupled with increased food intake to meet the energy demand. Neuropeptide Y may play a role in the appetitive phase of food intake (meal initiation), by drawing attention to food, and not the consummatory

phase (Seeley et al., 1995; Hillebrand et al., 2002a; Neary et al., 2004). Neary et al. (2004) reported a rapid increase in NPY before mealtimes in the PVN, and the levels remained elevated for as long as food was withheld.

Hypothalamic NPY neurones express leptin receptors, providing a means by which leptin regulates NPY expression (Mercer et al., 1996; Baskin et al., 1999). Elevated NPY mRNA expression has been reported in leptin knockout mice and genetic models of obesity, both linked to defective leptin signalling (Sanacora et al., 1990).

A.1.1.2 Agouti-related Protein

Agouti related peptide (AgRP) is a 132 AA peptide that is expressed only in the ARC and is a potent orexigenic peptide. A single picomolar dose centrally administered stimulated hyperphagia, lasting up to seven days in mice (Hagan et al., 2000) and transgenic mice that overexpress AgRP are obese and hyperphagic (Graham et al., 1997), Therefore the hypothesized function of AgRP in the CNS is to promote feeding and weight gain (Hagan et al., 2001).

The mechanisms through which AgRP stimulates feeding are unclear; but it is a potent antagonist of the melanocortin receptors, blocking the binding of α-MSH, which is an intake inhibitor (Yang et al., 1999). Experimental results indicate the feeding stimulatory effects of AgRP may involve NPY, as all AgRP terminals contain NPY (Broberger et al., 1998) and AgRP mRNA is extensively co-expressed in NPY neurons (Hahn et al., 1998). AgRP is up regulated when circulating concentrations of leptin are low, due to either fasting or mutation (Hahn et al., 1998; Wilson et al., 1999). Additionally, AgRP had been implicated as a central mediator of meal initiation because hypothalamic mRNA levels rise shortly before the onset of maximal daily food intake in ad libitum fed rats (Watson et al., 1999).

A.1.1.3 Orexins

The orexins are a class of orexigenic neuropeptides that were previously described as hypocretins. Orexin-a and b are 33- and 28-AA peptides, respectively, are localised in neurones in the dorsal and lateral hypothalamic regions (Sakurai et al., 1998). Orexins' actions are mediated through two receptors (Orexin-1 and Orexin-2) that are distributed within the brain (Sakurai et al., 1998). Both orexin-a and b stimulate food intake, however, it is the action of orexin a and receptor 1 that are the most potent (Sakurai et al., 1998).

The hyperphagic effects of orexin-a are thought to be mediated by NPY, as central administration of orexin increases NPY expression, but does not cause obesity (Yamanaka et al., 1999). Orexin mRNA is up regulated during states of fasting and hypoglycaemia (Cai et al., 1999). In comparison, orexin mRNA is reduced and food intake inhibited when leptin or α-MSH is centrally administered due to orexin neurons co-expressing leptin receptors (Lopez et al., 2000; Coll et al., 2007). Central administration of orexin-1 receptor antagonists suppress food intake and advance the onset of satiety, suggesting that orexin-a increases food intake by delaying the onset of satiety (Rodgers et al., 2002). In addition to stimulating food intake orexin-a is associated with wakefulness and arousal, as deletion of the orexin gene results in narcolepsy (Chemelli et al., 1999). These data suggest that orexin may be an important cellular and molecular link in the integration of sleep and energy homeostasis (Sakurai, 2003).

A.1.2 Anorexigenic Peptides

A.1.2.1 Melanocortin

Melanocortins (**MC**) are peptides that are cleaved from the precursor pro-opiomelanocortin (**POMC**), which is synthesized in specific neurones in the ARC and NTS, pituitary gland, and peripheral tissue (Bertagna, 1994; Castro and Morrison, 1997). Of the entire MC cleaved, α-MSH is considered the most important regulator of feeding (Williams et al., 2001). Central administration of α-MSH inhibit potently

inhibited feed intake and reduced body weight in mice indicating its anorexigenic role in feeding behaviour (Pierroz et al., 2002).

Three MC receptors have been identified within the brain, however, melanocortin-3 and 4- receptors, (MC-3 and MC-4) are expressed within the hypothalamic nuclei and are a key receptors underlying intake regulation and energy homeostasis (Pritchard et al., 2002), and both have a high affinity for α-MSH (Arora and Anubhuti, 2006).

The role of MC in feeding behaviour is strengthened by the presence of POMC neurons expressing the Ob-Rb leptin receptor in the ARC (Hillebrand et al., 2002a). When leptin is centrally administered, POMC neurons are stimulated (Cowley et al., 2001) increasing α-MSH leading to a decrease in food intake that is otherwise prevented by melanocortin antagonists (e.g. AgRP; Rossi et al., 1998; Yang et al., 1999). POMC expression is also decreased during early lactation in sheep (Sorensen et al., 2002) and rats (Smith, 1993; Pape and Tramu, 1996) promoting hyperphagia at a time when DMI is increasing to meet lactation demands.

A.1.2.2 Cocaine-and amphetamine-regulated transcript

Cocaine and amphetamine regulated transcript **(CART)** is a 116-AA expressed in several parts of the hypothalamus (ARC, PVN, DMH, LHA; Gautvik et al., 1996), as well as in the MeE, pituitary, and adrenal medulla (Kuhar and Dall Vechia, 1999).

Central administration of CART decreases normal and NPY-induced food intake (Kristensen et al., 1998; Lambert et al., 1998), and chronic CART administration decreases food intake and body weight (Larsen et al., 2000). CART expression is decreased during lactation in sheep perhaps by increased expression of AgRP and NPY to accommodate increasing intake during early lactation (Sorensen et al., 2002).

CART is hypothesised to be regulated by leptin as it is co-localized with leptin receptors (Kristensen et al., 1998; Larsen et al., 2000). Leptin knockout mice had reduced CART mRNA expression in the ARC, and central leptin administration increased CART expression (Kristensen et al., 1998). Food deprived rats had a decrease

in CART mRNA (Hillebrand et al., 2002a), suggesting that CART mRNA regulation is related to fuel availability and peripheral hormonal status (Li et al., 2002), as leptin decreases in fasted states (Myers, 2004).

Despite the effects of CART on food intake when centrally administered or in states of food deprivation, CART knockout mice have normal body weight and food intake (Bannon et al., 2000), therefore, its role in food intake is unclear.

A.2 Peripheral Intake Regulatory Signals

A.2.1 Gastro Intestinal

Most of the intake regulatory signals are sensitive to gut nutrient content and short-term feelings of hunger and satiety, mediated, in part, by coordinated changes in circulating hormone levels (Badman and Flier, 2005). Herein lies the difference between non-ruminant and ruminant species as

1. There is a constant influx of digesta entering the GI tract compared with discrete meals as in the non-ruminant.
2. Although glucose is the major fuel source in monogastric species, VFA are the primary fuel source in ruminants,
3. Absorption is confined mainly to the rumen, whereas in the non-ruminant absorption occurs in the small intestine.

Therefore, the secretion and function of gut-derived peptides may differ in the ruminant compared with monogastric species.

A.2.1.1 Orexigenic

A.2.1.1.1 Ghrelin

Ghrelin is a 28-amino acid (**AA**) peptide (27-AA) in bovine and ovine (Kojima et al., 2004) produced predominately in the oxyntic cells of the stomach (Kojima et al., 1999) and the abomasum in ruminant species (Hayashida et al., 2001). A total gasterectomy reduces plasma ghrelin by 60% (Small et al., 2009), indicating it is predominantly produced in the stomach. Among peptides, ghrelin is uniquely modified

by the addition of an octanoyl group to the serine residue at carbon position 3; this octanaylation makes ghrelin 'active' which is essential for binding to the G-protein coupled receptor (**GHS-R**) (Kojima et al., 1999).

Both centrally and peripherally administered ghrelin enhances food intake in rodents (Wren et al., 2000; Wren et al., 2001b), and humans (Wren et al., 2001a). Wertz-Lutz et al. (2006) reported an increase in feed intake in beef cows during the hour following subcutaneous infusion. Whereas, Roche et al. (2008b) continuously infused ghrelin via subcutaneous osmotic pumps for eight weeks in grazing dairy cows and did not report an increase in daily DM intake.

Peripheral ghrelin administration indicates ghrelins action within the ARC (Kojima et al., 1999; Horvath et al., 2001; Lu et al., 2002). Ninety percent of the NPY/AgRP neurons in the ARC express GHS-R, suggesting ghrelin may influence its effects via NPY/AgRP signalling (Willesen et al., 1999), with NPY as the primary effector (Chen et al., 2004).

Ghrelin is secreted in a pulsatile fashion (Bagnasco et al., 2002) and is regulated by nutritional status (Roche et al., 2008a). Circulating ghrelin concentrations increase in a fasted or pre fed state in both non-ruminant (Toshinai et al., 2001) and ruminant (Sugino et al., 2002a; Wertz-Lutz et al., 2006; Roche et al., 2007b) species, and decrease postprandially. The pre-prandial increases in ghrelin are hypothesised to regulate processes that are preparatory for the consumption and absorption of food.

Plasma ghrelin concentrations increased just prior to scheduled meal, in rats (Drazen et al., 2006), sheep (Sugino et al., 2002a) and humans (Cummings et al., 2001). However, Sugino et al. (2002a) reported that in unrestricted fed sheep pre-prandial surges were not evident and circulating ghrelin concentrations did not fluctuate during the day. Roche et al. (2007b) reported high ghrelin concentrations in unrestricted pasture fed dairy cows prior to consuming their a.m. supplement, followed by a decline in circulating ghrelin concentrations 2 hours after consuming a feed concentrate supplement and pasture, and that the decline was associated with the amount of concentrate consumed.

The postprandial decline in circulating ghrelin is reported to occur within 60 mins of consumption of a meal in both non-ruminant (Cummings et al., 2001; Callahan et al., 2004) and ruminant (Hayashida et al., 2001) species. Ghrelin concentration does not decline when water is ingested (Shiiya et al., 2002), indicating that gastric distension is not a regulating ghrelin suppression (Erdmann et al., 2003); instead ghrelin suppression post-prandially is related to the energy content and type of energy consumed.

1. Post-prandial ghrelin concentrations declined to a lower concentration when 33% of a total daily energy intake was ingested compared with 7.5 % total daily energy (Callahan et al., 2004).
2. Diets rich in carbohydrate decrease ghrelin concentrations more so than a fat-rich diet (Cummings et al., 2001; Erdmann et al., 2003; Callahan et al., 2004).

Ghrelin concentrations declined when lipids, amino acids and glucose were infused into the stomach, duodenum and jejunum (Overduin et al., 2005). However, the decrease in ghrelin concentrations upon glucose or amino acids infusion was substantially greater than when lipids were infused.

Murdolo et al. (2003) reported that changes in plasma insulin levels were associated with reciprocal changes in plasma ghrelin concentrations (i.e. hyperinsulemia supressed prandial ghrelin concentrations, whereas, absolute insulin deficiency prevented prandial plasma ghrelin suppression). The lower ghrelin suppression reported by Overduin et al. (2005) corresponded also to a low insulin response.

These data indicate that dietary nutrients either supress ghrelin directly, or indirectly through the corresponding increase in insulin secretion (Murdolo et al., 2003).

Plasma ghrelin concentrations have been reported to decline rapidly following intravenous administration of glucose in cows (Roche et al., 2008c) and humans (Shiiya et al., 2002; Nakai et al., 2003), indicating that luminal nutrient exposure in the stomach or duodenum, the principle sites of ghrelin production, is not required (Cummings, 2006). Associations between plasma ghrelin and glucose are low (Blom et

al., 2005) providing support for the hypothesis that ghrelin suppression is regulated by insulin.

A.2.1.1.2 Glucagon-like peptide 1

Glucagon-like peptide 1 (**GLP-1**) is an incretin hormone that is synthesized from L cells in the intestine in response to meal ingestion (Kieffer and Habener, 1999). The precursor to GLP-1 (7-36), GLP-1 (1-37), is cleaved from pro-glucagon, and the first six amino acids are removed from the N terminus to form the bioactive peptides GLP-1 (7-36) and GLP-1 (7-37), both of which stimulate insulin secretion (Orskov et al., 1993; Vahl et al., 2003). After release into circulation GLP-1 (7-36) is metabolized to GLP-1 (9-36) within 2 min by the protease enzyme dipeptidyl peptidase IV (DDP-IV) which is present on the endothelium of blood vessels (Mentlein et al., 1993; Deacon et al., 1995; Kieffer et al., 1995), representing the most abundant circulating form of GLP-1.

GLP-1 analogues interact with the GLP-1 receptor on the pancreatic β-cell (Orskov et al., 1993; Kieffer and Habener, 1999) closing ATP-dependent potassium channels in a glucose-dependent fashion, via protein kinase A; this is a pre-requisite for insulin secretion (Holz et al., 1993). The glucose dependency of the insulinotropic effects of GLP-1 ensures that insulin secretion is not stimulated in the presence of hypoglycaemia (Weir et al., 1989; Fehmann and Habener, 1992).

As well as its insulinotropic effects, GLP-1 inhibits gastric emptying (Flint et al., 1998; Meier et al., 2003), and reduces food intake (Turton et al., 1996; Gutzwiller et al., 1999). Intracerebroventricular (ICV) administration of GLP-1 inhibited feeding in fasted rats and at the beginning of the dark phase (Turton et al., 1996), which is when rats generally have increased feeding activity. A dose-dependent reduction in food intake was reported when GLP-1 was infused intravenously (Gutzwiller et al., 1999). However, when GLP-1 was administered intraperitoneally, there was no reduction in food intake (Turton et al., 1996), implying that GLP-1 action was through central rather than peripheral mechanisms. These data suggested that the mode site of peripheral infusion could influence GLP-1 feed inhibition effects.

Intravenous infusion of GLP-1 into 10-m-old steers resulted in an immediate increase in plasma insulin, and a decrease in plasma glucose (ThanThan et al., 2012). Faulkner and Pollock (1991) reported that GLP-1 increased the glucose-induced insulin response and accelerated plasma glucose clearance in starved sheep when GLP-1 was co-infused with glucose, indicating insulinotropic actions in ruminants under hyperglycaemic and normoglycemic conditions (ThanThan et al., 2012) similar to those reported in monogastric species.

GLP-1 concentrations were elevated in lactating compared with non-lactating sheep (Faulkner and Martin, 1997). This coincided with increased feed intake in the lactating sheep. However, there was no significant increase reported when the lactating sheep consumed 2.6 times more feed. Indicating that the GLP-1 increase reported in lactating sheep may not be attributed to an increase in feed intake. Faulkner and Martin (1997) concluded that the increased GLP-1 concentrations in the lactating sheep were attributed to increases over time and not at a particular meal.

A.2.1.1.3 Gastric Inhibitory Peptide

Gastric inhibitory polypeptide or glucose-dependent insulinotropic polypeptide (GIP) is secreted from the K cells in the duodenum and small intestine in response to glucose and fat absorption (Cataland et al., 1974). Gastric inhibitory polypeptide has been isolated from bovine small intestine (Carlquist et al., 1984). Once secreted into circulation in monogastric species, it acts on the pancreas to promote insulin secretion (McCarthy et al., 1992; Vilsbøll et al., 2003). Studies in sheep indicate that an increase in GIP is evident 1 hour after feeding, remaining elevated for 7 hours (McCarthy et al., 1992). Because over 60% of digestion occurs in the rumen (Merchen, 1988), it was hypothesised that the increase was not due to absorption of nutrients from the rumen, but instead the absorption of long-chain fatty acids in the small intestine (McCarthy et al., 1992). Despite its insulinotropic effects, GIP is not reported to have an acute influence on food intake (Murphy and Bloom, 2006).

A.2.1.1.4 Peptide YY

Peptide YY (**PYY**) is produced and secreted primarily from the L cells in the distal end of the GI tract (Adrian et al., 1985). Peptide YY is converted to its biologically active form PYY^{3-36} by the protease enzyme DDP-IV (Grandt et al., 1994). Plasma PYY concentrations increase to a plateau 1-2 h post ingestion, and are influenced by the number of calories and the composition of the meal (Wynne et al., 2004). Greater amounts of PYY are produced in response to a fat-rich meal than a carbohydrate or protein rich meal PYY is (Lin and Chey, 2003).

Initial studies on the action of PYY reported that peripheral administration delayed gastric emptying and gastric and pancreatic secretion, acting as an 'ileal brake' leading to the sensation of fullness However, additional studies have revealed that PYY effects are more related to reducing intake rather than inhibiting gastric emptying. Intraperitoneal PYY^{3-36} administration reduced dark phase and fasting induced feeding in rats without altering gastric emptying. This was also reported in humans, with a > 30% reduction in food intake 2 hours after PYY^{3-36} infusion; as was reported in rats, gastric emptying was not altered (Batterham et al., 2002). The satiety effects of PYY are mediated by inhibiting the NPY neurons and stimulating POMC expressing neurons expressed in the hypothalamus via the Y2 receptor (Grandt et al., 1996; Batterham et al., 2002).

A.2.1.1.5 Cholecystokinin

Cholecystokinin (CCK) is distributed throughout the GI tract and CNS, and is derived from a 95-AA pre-cursor peptide that undergoes post-translational or extracellular processing to yield multiple forms (Rehfeld, 1998). The various biologically active forms are characterised by the number of AA they contain. CCK-8 is the most abundant form in the human brain, while CCK-58, CCK-33, CCK-22, and CCK-8 are present in significant amounts in circulation and in intestines, with CCK-22 and CCK-33 the most abundant (Eberlein et al., 1988; Rehfeld et al., 2001). CCK has two receptors CCK-1R and CCK-2R (formally known as CCK_A and CCK_B). The CCK-1R is distributed within the GI tract, on the vagus nerve, and within the brain (Moran

and Kinzig, 2004), and has high- and low- affinity binding sites, that are adapted to respond to low or high concentrations of CCK (Pandya et al., 1994). CCK-2R is distributed in the CNS, on vagal afferents, and in the gastric mucosa, and has a high-affinity for the CCK-8 and CCK-5 (Baldwin et al., 1998). CCK is secreted from the intestine due to the presence of digestive products in the GI lumen, with dietary fat and protein being the most potent stimulators in monogastric species (Roche et al., 2008a). The actions of CCK include the stimulation of gallbladder contractions, bile (Liddle et al., 1985) and pancreatic exocrine secretion, inhibition of gastric secretion and slowing gastric emptying (Liddle et al., 1986), indicating its importance in the digestion of food.

In addition to its role in the digestion of food, CCK has been identified as an anorexigenic peptide. The intraperitoneal administration of CCK supressed intake of both solid and liquid food in a dose-dependent manner in rats (Gibbs et al., 1973). In humans, IV CCK administration increased the sensation of fullness, decreased hunger and subsequent energy intake, indicating the satiety effects of CCK (Kissileff et al., 1981; Muurahainen et al., 1988; Lieverse et al., 1994).

In ruminants, due to the delay between eating and the arrival of food at the duodenal CCK-producing sites, CCK might not have same effects as reported in monogastric species. Choi and Palmquist (1996) reported a dose-dependent increase in plasma CCK 3 hours post-feeding when increasing amounts of fat were fed to cows, suggesting a similar CCK response to dietary stimuli as in monogastric species. In contrast, Furuse et al. (1991) reported no significant fluctuations in plasma levels of CCK with either concentrate or roughage feeding, and Grovum (1981) intravenously infused CCK into sheep, and reported that CCK did not act directly on the central nervous system or the liver to supress food intake. Therefore, the role the CCK has in ruminants may differ from that reported in monogastric species

A.2.2 Pancreas

The pancreas is a gland adjacent to the proximal part of the duodenum and has both endocrine and exocrine roles. The exocrine portion of the pancreas produces sodium bicarbonate and digestive enzymes, which pass through the pancreatic ducts to empty into the duodenum close to the opening of the bile duct. The role for the

endocrine part of the pancreas is the maintenance of glucose homeostasis (Gomez-Ambrosi et al., 2009). Throughout the pancreas are small masses of endocrine tissue called pancreatic islets (formerly islets of Langerhans), making up 1-2% of the entire pancreas. The islets are highly vascularised allowing for the rapid response to changes in nutrients in the blood stream that signal the islets to secrete or not to secrete hormones (Kieffer and Habener, 2000). Distinct cell populations are still being discovered but the two best characterised are the α-cells and β-cells (Layden et al., 2010). Pancreatic hormones are first secreted into the portal circulation, and blood concentration represents the net difference between secretion rate and liver clearance (Harmon, 1992).

A.2.2.1 Insulin

Insulin is a 51-AA hormone secreted by the β-cells of the pancreatic islets. Insulin maintains glucose homeostasis by supressing hepatic glucose production (gluconeogenesis) and promoting glucose uptake by muscle and adipose tissue (Cryer, 2003). Beta cells are sensitive to increases in blood glucose, secreting insulin in response to increases in blood glucose. In addition to its glucose maintenance role, insulin is also secreted in proportion to the amount of stored fat, indicating it has a role as an adiposity signal (Bagdade et al., 1967; Polonsky et al., 1988). Insulin is the major endocrine stimulus for the state of anabolism that exists after a meal is digested and nutrients absorbed (Kieffer and Habener, 2000). Increases in plasma insulin suppress glucagon release from the α-cells, thus, preventing hepatic gluconeogenesis and lipolysis in the adipose tissue (Gomez-Ambrosi et al., 2009; Lafontan et al., 2009).

Horino et al. (1968) studied insulin production in both ruminant and non-ruminant species. When VFA were infused into non-ruminants there was no effect on insulin secretion; in comparison infused VFA increased insulin production without affecting glucose production in ruminant species. Additionally, when glucose was infused into sheep, insulin secretion increased but not to the same levels reported when VFA were infused. These results reported by Horino et al. (1968) highlight that, in ruminant species VFA are the major stimulator of insulin secretion rather than direct sources of glucose as in the non-ruminant.

In cattle and sheep, plasma insulin concentrations have been reported to increase significantly after feeding, with peaks observed 2 -4 hours after a meal (Brockman, 1978). However, plasma insulin correlate poorly with plasma glucose in the ruminant, instead, insulin is associated, with plasma VFA concentrations (Brockman, 1978). If the level of propionic acid absorbed exceeds the ability of the liver to convert to glucose, insulin production will be stimulated (Orskov, 1986). As the ruminant liver is capable of taking up only small amounts of glucose from blood (Ballard, 1965), the hepatic effects of insulin are less in the ruminant (Brockman, 1978). West and Passey (1967) and Brockman et al. (1975) reported that increased plasma insulin only supressed hepatic glucose production by 15% in sheep.

Insulin rapidly crosses the blood brain barrier by saturable receptor-mediated uptake in proportion to circulating concentrations (Margolis and Altszuler, 1967; Woods and Porte, 1977) and is evolving as a central regulator for satiety induction (Schwartz et al., 2000). Deetz and Wangsness (1981) reported an 18.5% decrease in total daily feed intake in sheep when physiological amounts of insulin were administered via IV injections. Intracerebroventricular administration of insulin inhibited food intake resulting in weight loss in rats (McGowan et al., 1992), baboons (Woods et al., 1979) and sheep (Foster et al., 1991). Inhibition of insulin signalling in the brain increased food intake, resulting in weight gain associated with peripheral insulin resistance (Air et al., 2002; Carvalheira et al., 2003). The anorexigenic effects of insulin are mediated by changes in the expression of hypothalamic neuropeptides (Plum et al., 2005). Central administration of insulin decreased expression of NPY (Schwartz et al., 1992; Sipols et al., 1995), increasing the expression of corticotropin-releasing hormone in the PVN (Sipols et al., 1995; Schwartz et al., 1996).

Insulin is considered a potential regulator of leptin. Hyperinsulinemia increased plasma leptin levels and gene expression in white adipose tissue in humans (Kolaczynski et al., 1996), rodents (Koopmans et al., 1998), and cows (Block et al., 2003; Lents et al., 2005), and plasma leptin levels return to normal when the insulinoma is removed (D'Adamo et al., 1998; Popovic et al., 1998). There is a reduction in plasma insulin accompanied by an increase in plasma glucose within 10 mins of leptin

administration in perfused rat islets cells (Kulkarni et al., 1997), indicating that leptin may be a regulatory factor in insulin secretion.

Additionally, to insulin increasing because of increasing glucose concentrations, insulin also increases within minutes of eating then decreases to levels pre-feeding and is characterised as the cephalic phase insulin response (**CPIR**). This response has been reported in non-ruminant (Powley and Berthoud, 1985; Teff, 2000) and ruminant (Vasilatos and Wangsness, 1980) species. The CPIR anticipates and mimics the post-absorptive insulin response (Power and Schulkin, 2008). Preventing the CPIR results in higher blood glucose concentrations and impairs the uptake of glucose for the first hour post prandial (Ahren and Holst, 2001). Administration of insulin immediately prior to a meal has been reported to improve glucose control in obese (Teff and Townsend, 1999) and type 2 diabetics (Bruttomesso et al., 1999). Results indicate that the CPIR appears to prime tissues in preparedness for the incoming absorbed nutrients.

A.2.2.2 Glucagon

Glucagon is a 29-AA hormone secreted by the α-cells of the pancreatic islets. Glucagon is involved in the regulation of glucose homeostasis through enhanced synthesis and mobilisation of glucose in the liver, and regulation of glucose-stimulated insulin secretion (Gomez-Ambrosi et al., 2009). Glucagon is released into circulation when circulating glucose is low, stimulating the liver to break down glycogen (glycogenolysis) and release glucose (gluconeogenesis) into circulation. Glucagon's actions provide the major counter–regulatory hormone opposing the actions of insulin in glucose homeostasis (Jiang and Zhang, 2003). Glucagon also stimulates adipocytes to release fatty acids (lipolysis) and increases the synthesis of glucose in the liver from substrates other than carbohydrates, such as amino acids (McGarry and Foster, 1980).

Glucagon is secreted immediately after eating (de Jong et al., 1977). The effect of glucagon can occur within minutes and dissipate rapidly (Dobbins et al., 1998), due to efficient hepatic clearance. The release of glucagon between meals exerts a positive effect on the pancreatic β-cells, acting as a 'primer' to enhance insulin release when plasma glucose concentrations are high (Pipeleers et al., 1985). The pancreatic β-cell

has glucagon receptors (Kieffer et al., 1996), which upon glucagon binding stimulates the increase of cAMP (Hussain et al., 2000). Levels of cAMP are crucial to nutrient sensing and subsequent insulin release (Philippe and Missotten, 1990; Oetjen et al., 1994). Pipeleers et al. (1985) reported low insulin release to nutrients in purified pancreatic β-cells and attributed response to the low cAMP levels. Insulin response to nutrient increased after cAMP or glucagon was added prior to nutrients (Pipeleers et al., 1985).

Different modes of administering glucagon (i.e. intravenous, intraperitoneal and intrahepatic) all reduce feeding (Geary, 1990, 1998). However, it is the hepatic portal infusions that elicit a rapid, dose-dependent decrease in food intake indicating that glucagon acts in the liver to inhibit eating (Woods et al., 2006). The signal to reduce meal size generated by glucagon reaches the brain via sensory axons of the vagus nerve (Woods et al., 2006). Deetz and Wangsness (1981) infused glucagon via intrajugular administration and reported a 15.8 % decrease in total daily food intake in sheep. She et al. (1999) reported that IV glucagon infusions for 12 days reduced the normal increases in feed intake of dairy cows postpartum. Although glucagon may act as a short-term anorexigenic factor, it is not a major regulator of long term feed intake in ruminant species (Roche et al., 2008a).

A.2.2.3 Pancreatic Polypeptide

Pancreatic polypeptide (PP) is a 36-AA peptide secreted by the F-cells in the pancreas and exerts a variety of regulatory functions, including modulation of gastric motility and pancreatic exocrine secretion. The post-prandial release of PP is biphasic with a small release to the first meal of the day, increasing with each subsequent meal (Wynne et al., 2004). The anorexigenic effects of PP might occur partly as a result of delayed gastric emptying (Murphy et al., 2006). However, the actions of PP differ depending on the species. For example, PP has an effect of gastric emptying in rodents but not in humans (Wynne et al., 2004). Depending on the route of administration, PP can have different effects on intake. Intracerebroventricular infusions into satiated rats stimulated daytime food intake (Clark et al., 1984). Whereas, when PP was peripherally administered into rats, gastric emptying and food intake was reduced (Asakawa et al.,

2003). These differences could be due to PP being unable to cross the BBB and reflect differing sites of receptor activation (Wynne et al., 2004).

A post-prandial increase in PP was reported 1 h after feeding and returned to pre-prandial concentrations within 3-6 h in cows (Choi and Palmquist, 1996). A dose-dependent increase in PP was reported, when supplementary fats were fed to cows, coincident with declining feed intake (Choi and Palmquist, 1996). As only pharmacological PP doses decrease feed intake, a physiological role for PP in the control of feed intake is uncertain.

A.2.3 Adipose Tissue

Adipose tissue represents an active endocrine organ that, in addition to regulating fat mass and nutrient homeostasis, releases a large number of adipokines (Rabe et al., 2008). As of 2009, more than 100 factors had been identified as produced and released by adipose tissue. Only a number of these are released into circulation in detectable or significant amounts (Hauner, 2009). Weight gain or obesity results in a greater secretion of some factors. For example, leptin is secreted in proportion to adipose tissue mass. If one were to discuss adipose tissue with regards to a role in regulating intake, only one factor would be prominent, leptin, although others do have a role in glucose homeostasis (Hauner, 2009).

A.2.3.1 Leptin

Leptin is a 146–AA protein hormone identified in 1994 (Zhang et al., 1994). It is primarily produced and secreted from adipose tissue, and in particular white adipose tissue, although it is also expressed at lower levels in the CNS and gastric epithelium (Bado et al., 1998). The leptin gene (also known as OB protein) is expressed in all adipose tissue, and the amount of circulating leptin is related to the amount of mRNA expressed (Prolo et al., 1998).

Six isoforms of the leptin receptor have been reported, and are characterised into three classes: long, short and secreted (Tartaglia, 1997). The long form differs from the other forms due to its active intracellular signalling domains (Tartaglia, 1997) which are

essential for leptin's action on intake (Lee et al., 1996). The long-form is mainly expressed in the ARC, PVN, DMH and LHA of the hypothalamus. The short-form of the leptin receptor aids in the saturable process of transporting leptin across the BBB (Banks et al., 1996), and the secreted form binds to circulating leptin, controlling its biological activity (Ge et al., 2002).

The central administration of leptin reduced food intake in ruminants (Morrison et al., 2001), decreasing food consumption over a 3 hour period by 63% and by 30% over a 24 h period in mice (Buettner et al., 2006). The site of leptin's action is in ARC of the hypothalamus via the long-form leptin receptor influencing two separate groups of neurons with opposing actions (Mercer et al., 1996). After binding to its receptor, leptin stimulates a signalling cascade that inhibits the orexigenic neuropeptide NPY/AgRP neurones while stimulating the anorexigenic POMC neurones to upregulate α-MSH inducing satiety (Jequier, 2002; Stanley et al., 2005). However, leptin concentration influences this cascade, with low levels up-regulating expression of the NPY/ AgRP neurones, by, inhibiting POMC neurons, and high leptin concentrations having the opposite effect (Stanley et al., 2005). Increasing concentrations signal the brain that excess energy is being stored, bringing about adaptations to decreases intake (Arora and Anubhuti, 2006). The regulation of leptin transport across the BBB is mediated by the level of food intake. Starvation reduces the transport of leptin across the BBB, while re-feeding has the opposite effect (Kastin and Pan, 2000).

Circulating leptin concentrations reflect both long-term energy stores and short-term changes in energy balance (Myers, 2004). Even though there is a high correlation with body fat mass, Delavaud et al. (2002) reported that plasma leptin was more strongly correlated with adipose cell size rather than the cows body condition score (**BCS**). When cows had to adjust to undernutrition there was not a relationship between leptin and BCS, whereas the relationship with adipose cell size were still strongly related (Delavaud et al., 2002).

Leptin secretion in women follows circadian rhythm, with a nadir early in the morning (0800-0900 h), an increase during the day, and a peak between 2400 and 0200 h (Licinio et al., 1998). After the last meal of the day, leptin increases to a plateau

around 2200 h and hypothesised to reflect overall accumulation of ingested calories (Cummings et al., 2001). A circadian rhythm for leptin secretion has not been observed in ruminants (Daniel et al., 2002).

Short-term food restriction can also suppress circulating leptin acutely, which can be reversed by re-feeding (Small et al., 2009). The regulation of leptin secretion by acute changes in energy balance indicates the adipose tissue response to circulating hormones or metabolite's that are affected by energy intake, for example insulin. Leptin expression increases after peak insulin secretion and insulin directly stimulates leptin expression in adipocyte cultures (Ahima and Flier, 2000). Leptin is decreased in response to insulin deficiency and increased in response to insulin treatment (Ahima et al., 2000). Insulin is a positive regular of plasma leptin in lactating cows when in positive energy balance and suggests that reductions in plasma insulin during periods of nutritional deficit could be responsible for mediating a portion of the coincidental decrease in plasma leptin (Block et al., 2003).

A.2.3.2 Adiponectin

Adiponectin is a 244-AA protein secreted primarily by the white and brown adipose tissue (Kershaw and Flier, 2004). Adiponectin exerts its effects through binding to at least three receptors. The first two receptors AdipoR1 and AdipR2 are mainly expressed in skeletal and liver tissue, respectively (Gomez-Ambrosi et al., 2009). The third receptor T-cadherin is thought to act as a co-receptor to transmit metabolic signals, but its functional implications are yet to be determined (Hug et al., 2004). An adiponectin receptor has yet to be discovered centrally (Kadowaki and Yamauchi, 2005); therefore it is unlikely that adiponectin has direct effects on the regulation of intake. Peripheral administration of adiponectin in rodents stimulated energy expenditure and reduced body weight gain, without any change in intake (Kadowaki and Yamauchi, 2005). Adiponectin administration improved glucose uptake in the peripheral tissue of rodents (Yamauchi et al., 2003). Circulating adiponectin is inversely correlated with insulin resistance (i.e. lower in obese individuals (Arita et al., 1999) and higher after weight loss (Yang et al., 2001). Adiponectin, therefore, may

play an indirect role in intake through changes in circulating insulin and glucose (Roche et al., 2008a).

A.2.3.3 Resistin

Resistin is a 92-AA peptide that is highly expressed in the adipose tissue and secreted into circulation in mice (Gomez-Ambrosi et al., 2009), and expressed at lower levels in human adipose tissue (Gomez-Ambrosi and Fruhbeck, 2005). Under physiological conditions, resistin opposes insulins action in adipocytes and impairs glucose tolerance and insulin sensitivity in mice (Gomez-Ambrosi et al., 2009). Insulin-stimulated glucose uptake was enhanced in adipocytes in resistin knockout studies, and was reduced upon resistin treatment (Steppan et al., 2001). Resistin has been reported to have similar effects in the dairy cow as the expression of resistin in adipose tissue is greater in the lactating cow and insulin resistance is high, than the non-lactating cow (Komatsu et al., 2003). With this in mind the exact role of resistins involvement in the development of insulin resistance, still needs to be determined (McTernan et al., 2006).

References

Abbott, N. J. 1992. Comparative physiology of the blood-brain barrier. In: Physiology and Pharmacology of the Blood-Brain Barrier. M. W. B. Bradbury. Springer, Heidelberg. pp 371-396,

Adrian, T. E., G. L. Ferri, A. J. Bacarese-Hamilton, H. S. Fuessl J. M. Polak, and S. R. Bloom. 1985. Human distribution and release of a putative new gut hormone, peptide YY. Gastroenterology 89: 1070-1077.

AFRC. 1998. Responses in the yield of milk constituents to the intake of nutrients by dairy cows. CAB Int., Wallingford.

Ahima, R. S., and D. A. Antwi. 2008. Brain Regulation of Appetite and Satiety. Endocrinology and Metabolism Clinics of North America 37: 811-823.

Ahima, R. S. and J. S. Flier. 2000. Leptin. Annual Review of Physiology 62: 413-437.

Ahima, R. S., D. Prabakaran, C. Mantzoros, D. Qu, B. Lowell, E. Maratos-Flier, and J. S. Flier. 1996. Role of leptin in the neuroendocrine response to fasting. Nature 382: 250-252.

Ahima, R. S., C. B. Saper, J. S. Flier, and J. K. Elmquist. 2000. Leptin regulation of neuroendocrine systems. Frontiers in Neuroendocrinology 21: 263-307.

Ahlman, H. and O. Nilsson. 2001. The gut is the largest endocrine organ in the body. Annals of Oncology 12: 63-68.

Ahren, B. and J. J. Holst. 2001. The cephalic insulin response to meal ingestion in humans is dependent on both cholinergic and non-cholinergic mechanisms and is important for postprandial glycaemia. Diabetes 50: 1030-1038.

Air, E. L., M. Z. Strowski, S. C. Benoit, S. L. Conarello, G. M. Salituro, X. M. Guan, K. Liu, S. C. Woods, and B. B. Zhang. 2002. Small molecule insulin mimetics reduce food intake and body weight and prevent development of obesity. Nature Medicine 8: 179-183.

Akin, D. E., G. L. R. Gordon, and J. P. Hogan. 1983. Rumen bacterial and fungal degradation of *Digitaria pentzii* grown with or without sulphur. Applied and Environmental Microbiology 46: 738-748.

Albright, J. L. 1993. Feeding behaviour of dairy cattle. Journal of Dairy Science 76: 485-498.

Allen, A., and A. Garner. 1980. Mucus and bicarbonate secretion in the stomach and their possible role in mucosal protection. Gut 21: 249-262.

Allen, J. M., T. E. Adrian, J. M. Polak, and S. R. Bloom. 1983a. Neuropeptide Y (NPY) in the adrenal gland. Journal of the Autonomic Nervous System 9: 559-563.

Allen, J. M., P. M. Bircham, S. R. Bloom, and A. V. Edwards. 1984. Release of neuropeptide Y in response to splanchnic nerve stimulation in the conscious calf. The Journal of Physiology 357: 401-408.

Allen, M. S. 1996. Physical constraints on voluntary intake of forages by ruminants. Journal of Animal Science 74: 3063-3075.

Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. Journal of Dairy Science 83: 1598-1624.

Allen, M. S. and B. J. Bradford. 2009. HOT feeding strategies to maximize milk yield. Four-State Dairy Nutrition and Management Conference, Dubuque, Iowa.

Allen, M. S., B. J. Bradford, and M. Oba. 2009. Board Invited Review: The hepatic oxidation theory of the control of feed intake and its application to ruminants. Journal of Animal Science 87: 3317-3334.

Allen, M. S. and M. Oba. 1996. Fibre digestibility of forages. In: Proceedings of the 57th Minnesota Nutrition Conference Protiva Technical Symposium Extension Special Programs, University of Minnesota, St. Paul, USA. pp 151-171.

Allen, Y. S., T. E. Adrian, J. M. Allen, K. Tatemoto, T. J. Crow, S. R. Bloom, and J. M. Polak. 1983. Neuropeptide Y distribution in the rat brain. Science 221: 877-879.

Anderson, K. A., T. J. Ribar, F. Lin, P. K. Noeldner, M. F. Green, M. J. Muehlbauer, L. A. Witters, B. E. Kemp, and A. R. Means. 2008. Hypothalamic CaMKK2 contributes to the regulation of energy balance. Cell Metabolism 7: 377-388.

Annison, E. F. and D. B. Lindsay. 1961. Acetate utilization in sheep. Journal of Biochemistry 78: 777-785.

Arita, Y., S. Kihara, N. Ouchi, M. Takahashi, K. Maeda, J. Miyagawa, K. Hotta, I. Shimomura, T. Nakamura, K. Miyaoka, H. Kuriyama, M. Nishida, S. Yamashita, K. Okubo, K. Matsubara, M. Muraguchi, Y. Ohmoto, T. Funahashi, and Y. Matsuzawa. 1999. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochemical and Biophysical Research Communications 257: 79-83.

Armentano, L. E. 1992. Ruminant hepatic metabolism of volatile fatty acids, lactate and pyruvate. The Journal of Nutrition 122: 838-842.

Arora, S., and Anubhuti. 2006. Role of neuropeptides in appetite regulation and obesity. Neuropeptides 40: 375-401.

Arosio, M., C. L. Ronchi, P. Beck-Peccoz, C. Gebbia, C. Giavoli, V. Cappiello, D. Conte, and M. Peracchi. 2004. Effects of modified sham feeding on ghrelin levels in healthy human subjects. Journal of Clinical Endocrinology and Metabolism 89: 5101-5104.

Asakawa, A., A. Inui, H. Yuzuriha, N. Ueno, G. Katsuura, M. Fujimiya, M. A. Fujino, A. Niijima, M. M. Meguid, and M. Kasuga. 2003. Characterization of the effects of pancreatic polypeptide in the regulation of energy balance. Gastroenterology 124: 1325-1336.

Ash, R. W. 1961. Acid secretion by the abomasum and its relation to the flow of food material in sheep. Journal of Physiology 156: 93-111.

Badman, M. K. and J. S. Flier. 2005. The gut and energy balance: visceral allies in the obesity wars. Science 307: 1909-1914.

Bado, A., S. Levasseur, S. Attoub, S. Kermorgant, J. P. Laigneau, M. N. Bortoluzzi, L. Moizo, T. Lehy, M. Guerre-Millo, Y. Le Marchand-Brustel, and M. J. Lewin. 1998. The stomach is a source of leptin. Nature 394: 790-793.

Bagdade, J. D., E. L. Bierman, and D. Porte, Jr. 1967. The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. Journal of Clinical Investigations 46: 1549-1557.

Bagnasco, M., P. S. Kalra, and S. P. Kalra. 2002. Ghrelin and leptin pulse discharge in fed and fasted rats. Endocrinology 143: 726-729.

Baird, G. D. 1977. Aspects of ruminant intermediary metabolism in relation to ketosis. Biochemical Society Transactions 5: 819-827.

Baird, G. D., M. A. Lomax, H. W. Symonds, and S. R. Shaw. 1980. Net hepatic and splanchnic metabolism of lactate, pyruvate and propionate in dairy cows in vivo in relation to lactation and nutrient supply. Journal of Biochemistry 186: 47-57.

Baird, G. D., H. W. Symonds, and R. Ash. 1975. Some observations on metabolite production and utilization in vivo by the gut and liver of adult dairy cows. Journal of Agricultural Science 85: 281-296.

Baker, R. D., L. P. Le Du, and F. Alvarez. 1981. The herbage intake and performance of set-stocked suckler cows and calves. Grass and Forage Science 36: 201-210.

Baldwin, B. A., R. F. Parrott, and I. S. Ebenezer. 1998. Food for thought: a critique on the hypothesis that endogenous cholecystokinin acts as a physiological satiety factor. Progress in Neurobiology 55: 477-507.

Ballard, F. J. 1965. Glucose utilization in mammalian liver. Comparative Biochemistry and Physiology 14: 437-443.

Banks, W. A., A. J. Kastin, W. Huang, J. B. Jaspan, and L. M. Maness. 1996. Leptin enters the brain by a saturable system independent of insulin. Peptides 17: 305-311.

Bannink, A., J. France, S. Lopez, W. J. J. Gerrits, E. Kebreab, S. Tamminga, and J. Dijkstra. 2008. Modelling the implications of feeding strategy on rumen fermentation and functioning of the rumen wall. Animal Feed Science and Technology 143: 3-26.

Bannon, A. W., J. Seda, M. Carmouche, W. G. Richards, W. Fan, M. Jarosinski, A. A. McKinzie, and J. Douglas. 2000. Biological functions of cocaine- and amphetamine-regulated transcipt (CART): data with CART peptides and CART knockout mice. Society of Neuroscience 26: 2041.

Bargo, F., L. D. Muller, J. E. Delahoy, and T. W. Cassidy. 2002. Performance of high producing dairy cows with three different feeding systems combining pasture and total mixed rations. Journal of Dairy Science 85: 2948-2963.

Bargo, F., L. D. Muller, E. S. Kolver, and J. E. Delahoy. 2003. Invited review: Production and digestion of supplemented dairy cows on pasture. Journal of Dairy Science 86: 1-42.

Barker-Gibb, M. L. and I. J. Clarke. 1996. Increased galanin and neuropeptide-Y immunoreactivity within the hypothalamus of ovariectomised ewes following a prolonged period of reduced body weight is associated with changes in plasma growth hormone but not gonadotropin levels. Neuroendocrinology 64: 194-207.

Bartley, E. E. 1976. Bovine saliva: Production and function. Buffers in Ruminant Physiology and Metabolism. Church and Dwight Company Inc. , New York.

Baskin, D. G., T. M. Hahn, and M. W. Schwartz. 1999. Leptin sensitive neurons in the hypothalamus. Hormone and Metabolic Research 31: 345-350.

Batterham, R. L., M. A. Cowley, C. J. Small, H. Herzog, M. A. Cohen, C. L. Dakin, A. M. Wren, A. E. Brynes, M. J. Low, M. A. Ghatei, R. D. Cone, and S. R. Bloom. 2002. Gut hormone PYY (3-36) physiologically inhibits food intake. Nature 418: 650-654.

Bauchart, D. 1993. Lipid absorption and transport in ruminants. Journal of Dairy Science 76: 3864-3881.

Bauchop, T. 1979a. Rumen anaerobic fungi of cattle and sheep. Applied Environmental Microbiology 38: 148-158.

Bauchop, T. 1979b. The rumen anaerobic fungi; colonizers of plant fibre. Annals of Veterinary Research 10: 246-248.

Bauchop, T. 1981. The anaerobic fungi in rumen fibre digestion. Agriculture and Environment 6: 339-348.

Baumgardt, B. R. 1969. Utilization of cellulose by ruminants. Cellulases and their Applications. American Chemical Society, America.

Bergman, E. N. 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiological Reviews 70: 567-590.

Bergman, E. N., and J. E. Wolff. 1971. Metabolism of volatile fatty acids from the gastrointestinal tract in various species. American Journal of Physiology 221: 586-592.

Bertagna, X. 1994. Proopiomelanocortin-derived peptides. Endocrinology and Metabolism Clinics of North America 23: 467-485.

Billington, C. J., J. E. Briggs, M. Grace, and A. S. Levine. 1991. Effects of intracerebroventricular injection of neuropeptide Y on energy metabolism. The American Journal of Physiology 260: 321-327.

Bines, J. A. and S. V. Morant. 1983. The effect of body condition on metabolic changes associated with intake of food by the cow. British Journal of Nutrition 50: 81-89.

Bird, S. H. and R. A. Leng. 1978. The effects of defaunation of the rumen on the growth of cattle on low-protein high-energy diets. British Journal of Nutrition 49: 163-167.

Blache, D., R. Tellam, L. Chagas, M. Blackberry, P. Vercoe, and G. Martin. 2000. Level of nutrition affects leptin concentrations in plasma and cerebrospinal fluid in sheep. Journal of Endocrinology 165: 625-637.

Black, A. L., M. Kleiber, and A. M. Brown. 1961. Butyrate metabolism in the lactating cow. Journal of Biological Chemistry 236: 2399-2403.

Block, S. S., R. P. Rhoads, D. E. Bauman, R. A. Ehrhardt, M. A. McGuire, B. A. Crooker, J. M. Griinari, T. R. Mackle, W. J. Weber, M. E. Van Amburgh, and Y. R. Boisclair. 2003. Demonstration of a role for insulin in the regulation of leptin in lactating dairy cows. Journal of Dairy Science 86: 3508-3515.

Blom, W. A., A. Stafleu, C. de Graaf, F. J. Kok, G. Schaafsma, and H. F. Hendriks. 2005. Ghrelin response to carbohydrate-enriched breakfast is related to insulin. American Journal of Clinical Nutrition 81: 367-375.

Bodosi, B., J. Gardi, I. Hajdu, E. Szentirmai, F. Obal, and J. M. Krueger. 2004. Rhythms of ghrelin, leptin and sleep in rats: effects of the normal diurnal cycle, restricted feeding, and sleep deprivation. American Journal of Physiology, Regulatory, Integrative and Comparative Physiology 287: 1071-1079.

Bost, J. 1969. Omasal physiology. In: Physiology of digestion and metabolism in the ruminant. Proceedings of the 3rd international symposium, Cambridge, England. pp. 52-65

Broberger, C. 2005. Brain regulation of food intake and appetite: molecules and networks. Journal of Internal Medicine 258: 301-327.

Broberger, C., J. Johansen, C. Johansson, M. Schalling, and T. Hokfelt. 1998. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic and monosodium glutamate-treated mice. Proceedings of the National Academy of Sciences of the United States of America 95: 15043-15048.

Broberger, C., M. Landry, H. Wong, J. N. Walsh, and T. Hokfelt. 1997. Subtypes Y1 and Y2 of the neuropeptide Y receptor are respectively expressed in pro-opiomelanocortin- and neuropeptide-Y-containing neurons of the rat hypothalamic arcuate nucleus. Neuroendocrinology 66: 393-408.

Brockman, R. P. 1978. Roles of glucagon and insulin in the regulation of metabolism in ruminants. Canadian Veterinary Journal 19: 55-62.

Brockman, R. P. 2005. Glucose and short-chain fatty acid metabolism. Quantitative aspects of Ruminant Digestion and Metabolism. CABI Int, Oxfordshire, UK.

Brockman, R. P., E. N. Begman, W. L. Pollak, and J. Brondum. 1975. Studies of glucose production in sheep using (6-^{3}H) glucose and (U-^{14}C) glucose. Canadian Journal of Physiology and Pharmacology 53: 1186-1189.

Bruttomesso, D., A. Pianta, A. Mari, A. Valerio, M. C. Marescotti, A. Avogaro, A. Tiengo, and S. Del Prato. 1999. Restoration of early rise in plasma insulin levels improves the glucose tolerance of type 2 diabetic patients. Diabetes 48: 99-105.

Bryant, M. P. and N. Small. 1955. Characteristics of two new genera of anaerobic curved rods isolated from the rumen of cattle. Journal of Bacteriology 72: 16-26.

Buettner, C., A. Pocai, E. D. Muse, A. M. Etgen, M. G. Myers, Jr., and L. Rossetti. 2006. Critical role of STAT3 in leptin's metabolic actions. Cell Metabolism 4: 49-60.

Bugaut, M. 1987. Occurrence, absorption and metabolism of short chain fatty acids in the digestive tract of mammals. Comparative Biochemistry and Physiology 86:439-472.

Butler, W. R., and R. D. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. Journal of Dairy Science 72: 767-783.

Cai, X. J., P. S. Widdowson, J. Harrold, S. Wilson, R. E. Buckingham, J. R. Arch, M. Tadayyon, J. C. Clapham, J. Wilding, and G. Williams. 1999. Hypothalamic orexin expression: modulation by blood glucose and feeding. Diabetes 48: 2132-2137.

Callahan, H. S., D. E. Cummings, M. S. Pepe, P. A. Breen, C. C. Matthys, and D. S. Weigle. 2004. Postprandial suppression of plasma ghrelin level is proportional to ingested caloric load but does not predict intermeal interval in humans. Journal of Clinical Endocrinology and Metabolism 89: 1319-1324.

Carlquist, M., M. Maletti, H. Jornvall, and V. Mutt. 1984. A novel form of gastric inhibitory polypeptide (GIP) isolated from bovine intestine using a radioreceptor assay. Fragmentation with staphylococcal protease results in GIP1-3 and GIP4-42, fragmentation with enterokinase in GIP1-16 and GIP17-42. European Journal of Biochemistry 145: 573-577.

Carro, M. D., C. Valdes, M. J. Ranilla, and J. S. Gonzalez. 2000. Effect of forage to concentrate ratio in the diet on ruminal fermentation and digesta flow kinetics in sheep offered food at a fixed and restricted level of intake. Journal of Animal Science 70: 127-134.

Carroll, E. J. and R. E. Hungate. 1954. The magnitude of the microbial fermentation in the bovine rumen. Journal of Applied Microbiology 2: 205-214.

Carvalheira, J. B., E. B. Ribeiro, E. P. Araujo, R. B. Guimaraes, M. M. Telles, M. Torsoni, J. A. Gontijo, L. A. Velloso, and M. J. Saad. 2003. Selective impairment of insulin signalling in the hypothalamus of obese zucker rats. Diabetologia 46: 1629-1640.

Castro, M. G., and E. Morrison. 1997. Post-translational processing of proopiomelanocortin in the pituitary and in the brain. Critical Reviews in Neurobiology 11: 35-57.

Cataland, S., S. E. Crockett, J. C. Brown, and E. L. Mazzaferri. 1974. Gastric inhibitory polypeptide (GIP) stimulation by oral glucose in man. The Journal of Clinical Endocrinology and Metabolism 39: 223-228.

Chagas, L. M., M. C. Lucy, P. J. Back, D. Blache, J. M. Lee, P. J. S. Gore, A. J. Sheahan, and J. R. Roche. 2009. Insulin resistance in divergent strains of holstein-friesian dairy cows offered fresh pasture and increasing amounts of concentrate in early lactation. Journal of Dairy Science 92: 216-222.

Chemelli, R. M., J. T. Willie, C. M. Sinton, J. K. Elmquist, T. Scammell, C. Lee, J. A. Richardson, S. C. Williams, Y. Xiong, Y. Kisanuki, T. E. Fitch, M. Nakazato, R. E. Hammer, C. B. Saper, and M. Yanagisawa. 1999. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 98: 437-451.

Chen, H. Y., M. E. Trumbauer, A. S. Chen, D. T. Weingarth, J. R. Adams, E. G. Frazier, Z. Shen, D. J. Marsh, S. D. Feighner, X. M. Guan, Z. Ye, R. P. Nargund, R. G. Smith, L. H. Van der Ploeg, A. D. Howard, D. J. MacNeil, and S. Qian. 2004. Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. Endocrinology 145: 2607-2612.

Chilibroste, P., S., Tamminga, and H. Boer. 1997. Effects of length of grazing session, rumen fill and starvation time before grazing on dry-matter intake, ingestive behaviour and dry-matter rumen pool sizes of grazing lactating dairy cows. Grass and Forage Science 52: 249-257.

Choi, B. R. and D. L. Palmquist. 1996. High fat diets increase plasma cholecystokinin and pancreatic polypeptide, and decrease plasma insulin and feed intake in lactating cows. The Journal of Nutrition 126: 2913-2919.

Church, D. C. 1993. The Ruminant Animal: Digestive Physiology and Nutrition. Waveland Press, Inc, Illinois, USA.

Clark, J. T., P. S. Kalra, W. R. Crowley, and S. P. Kalra. 1984. Neuropeptide Y and human pancreatic polypeptide stimulate feeding behaviour in rats. Endocrinology 115: 427-429.

Clutton-Brock, J. 1999. A Natural History of Domesticated Mammals. Cambridge University Press, Cambridge, UK.

Cohen-Bazire, G., G. Cohen, and A. Prévot. 1948. Nature et mode de formation des acides volatils dans les cultures de quelques bactéries anaérobies protéolytiques du groupe de Cl. sporogenes. Formation par réaction de Stickland des acides isobutyrique, isovalérianique et valérianique optiquement actifs. Annales de l'Institut Pasteur 75: 291-304.

Coleman, S. W. and K. M. Barth. 1977. Utilization of supplemental NPN and energy sources by beef steers consuming low-protein hays. Journal of Animal Science 45: 1180-1187.

Coll, A. P., I. S. Farooqi, and S. O'Rahilly. 2007. The hormonal control of food intake. Cell 129: 251-262.

Collin, M., M. Bäckberg, M.-L. Ovesjö, G. Fisone, R. H. Edwards, F. Fujiyama, and B. Meister. 2003. Plasma membrane and vesicular glutamate transporter mRNAs/proteins in hypothalamic neurons that regulate body weight. European Journal of Neuroscience 18: 1265-1278.

Cork, S. J. 1996. Optimal digestive strategies for arboreal herbivorous mammals in contrasting forest types: Why Koalas and Colobines are different. Australian Journal of Ecology 21: 10-20.

Cowley, M. A., N. Pronchuk, W. Fan, D. M. Dinulescu, W. F. Colmers, and R. D. Cone. 1999. Integration of NPY, AGRP, and melanocortin signals in the hypothalamic paraventricular nucleus: evidence of a cellular basis for the adipostat. Neuron 24: 155-163.

Cowley, M. A., J. L. Smart, M. Rubinstein, M. G. Cerdan, S. Diano, T. L. Horvath, R. D. Cone, and M. J. Low. 2001. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature 411: 480-484.

Cronje, P. B., J. V. Nolan, and R. A. Leng. 1991. Acetate clearance rate as a potential index of the availability of glucogenic precursors in ruminants fed on roughage-based diets. British Journal of Nutrition 66: 301-312.

Cryer, P. E. 2003. Glucose homeostasis and hypoglycemia. Williams Textbook of Endocrinology. Saunders, Pennsylvania. USA.

Cummings, D. E. 2006. Ghrelin and the short- and long-term regulation of appetite and body weight. Physiology and Behaviour 89: 71-84.

Cummings, D. E., R. S. Frayo, C. Marmonier, R. Aubert, and D. Chapelot. 2004. Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. American Journal of Physiology. Endocrinology and Metabolism 287: 297-304.

Cummings, D. E., J. Q. Purnell, R. S. Frayo, K. Schmidova, B. E. Wisse, and D. S. Weigle. 2001. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50: 1714-1719.

Czerkawski, J. W. 1986. Degradation of solid feeds in the rumen; spatial distribution of microbial activity and its consequences. Control of Digestion and Metabolism in Ruminants. Proceedings of the Sixth international Symposium on Ruminant Physiology. Prentice Hall, New Jersey.

D'Adamo, M., A. Buongiorno, E. Maroccia, F. Leonetti, F. Barbetti, A. Giaccari, D. Zorretta, G. Tamburrano, and P. Sbraccia. 1998. Increased OB gene expression leads to elevated plasma leptin concentrations in patients with chronic primary hyperinsulinemia. Diabetes 47: 1625-1629.

Dado, R. G. and M. S. Allen. 1994. Variation in and relationships among feeding, chewing, and drinking variables for lactating dairy cows. Journal of Dairy Science 77: 132-144.

Dalley, D. E., J. R. Roche, P. J. Moate, and C. Grainger. 2001. More frequent allocation of herbage does not improve the milk production of dairy cows in early lactation. Australian Journal of Experimental Agriculture 41: 593-599.

Danfaer, A. 1994. Nutrient metabolism and utilization in the liver. Livestock Production Science 111: 201-210.

Daniel, J. A., B. K. Whitlock, J. A. Baker, B. Steele, C. D. Morrison, D. H. Keisler, and J. L. Sartin. 2002. Effect of body fat mass and nutritional status on 24-hour leptin profiles in ewes. Journal of Animal Science 80: 1083-1089.

Date, Y., N. Murakami, K. Toshinai, S. Matsukura, A. Niijima, H. Matsuo, K. Kangawa, and M. Nakazato. 2002. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. Gastroenterology 123: 1120-1128.

De Jong, A., J. H. Strubbe, and A. B. Steffens. 1977. Hypothalamic influence on insulin and glucagon release in the rat. American Journal of Physiology 233: 380-388.

Deacon, C. F., A. H. Johnsen, and J. J. Holst. 1995. Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. The Journal of Clinical Endocrinology and Metabolism 80: 952-957.

Decavel, C. and A. N. Van den Pol. 1990. GABA: a dominant neurotransmitter in the hypothalamus. The Journal of Comparative Neurology 302: 1019-1037.

Deetz, L. E. and P. J. Wangsness. 1981. Influence of intrajugular administration of insulin, glucagon and propionate on voluntary feed intake of sheep. Journal of Animal Science 53: 427-433.

DeFronzo, R. A. and D. Tripathy. 2009. Skeletal Muscle Insulin Resistance Is the Primary Defect in Type 2 Diabetes. Diabetes Care 32: 157-163.

Dehority, B. A. 1991. Cellulose digestion in ruminants. Biosynthesis and Biodegradation of Cellulose. Marcel Dekker, New York.

Delaby, L., J. L. Peyraud, and R. Delagarde. 2001. Effect of the level of concentrate supplementation, herbage allowance and milk yield at turn-out on the performance of dairy cows in mid lactation at grazing. Animal Science 73: 171-181.

Delavaud, C., A. Ferlay, Y. Faulconnier, F. Bocquier, G. Kann, and Y. Chilliard. 2002. Plasma leptin concentration in adult cattle: effects of breed, adiposity, feeding level, and meal intake. Journal of Animal Science 80: 1317-1328.

Demment, M. W., E. A. Laca, and G. B. Greenwood. 1986. Intake in grazing ruminants: A conceptual framework In: Feed Intake by Beef Cattle. F. N. Owens. Agricultural Experiment Station MP-121, Oklahoma. pp 208-225

Dijkstra, J., H. Boer, J. Van Bruchem, M. Bruining, and S. Tamminga. 1993. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. British Journal of Nutrition 69: 385-396.

Dijkstra, J., and S. Tamminga. 1995. Simulation of the effects of diet on the contribution of rumen protozoa to degradation of fibre in the rumen. British Journal of Nutrition 74: 617-634.

Dillon, P. 2006. Achieving high dry-matter intake from pasture with grazing dairy cows. In: Fresh Herbage for Dairy Cattle. Springer. The Netherlands, pp 1-26.

Dillon, P. and F. Buckley. 1998. Managing and feeding high genetic merit dairy cows at pasture. R&H Hall Technical Bulletin. Issue No. 2

Diouf, J. 1995. World Animal Review. 84-85: 83.

Dixon, R. M. and C. R. Stockdale. 1999. Associative effects between forages and grains: consequences for feed utilisation. Australian Journal of Agriculture Research 50: 757-773.

Dobbins, R. L., S. N. Davis, D. Neal, A. Caumo, C. Cobelli, and A. D. Cherrington. 1998. Rates of glucagon activation and deactivation of hepatic glucose production in conscious dogs. Metabolism: Clinical and Experimental 47: 135-142.

Dobson, D. E., E. M. Prager, and A. C. Wilson. 1984. Stomach lysozymes of ruminants. The Journal of Biological Chemistry 259: 11607-11616.

Domingue, B. M. F., D. W. Dellow, and T. N. Barry. 1991. Voluntary intake and rumen digestion of a low-quality roughage by goats and sheep. The Journal of Agricultural Science 117: 111-120.

Downing, J. A., J. Joss, P. Connell, and R. J. Scaramuzzi. 1995. Ovulation rate and the concentrations of gonadotrophic and metabolic hormones in ewes fed lupin grain. Journal of Reproduction and Fertility 103: 137-145.

Drapanas, T., J. S. Williams, J. C. McDonald, W. Heyden, T. Bow, and R. P. Spencer. 1963. Role of the ileum in the absorption of vitamin b_{12} and intrinsic factor. The Journal of the American Medical Association 184: 337-341.

Drazen, D. L., T. P. Vahl, D. A. D'Alessio, R. J. Seeley, and S. C. Woods. 2006. Effects of a fixed meal pattern on ghrelin secretion: evidence for a learned response independent of nutrient status. Endocrinology 147: 23-30.

Dzaja, A., M. A. Dalal, H. Himmerich, M. Uhr, T. Pollmächer, and A. Schuld. 2004. Sleep enhances nocturnal plasma ghrelin levels in healthy subjects. American Journal of Physiology, Endocrinology and Metabolism 286: 963-967.

Eberlein, G. A., V. E. Eysselein, and H. Goebell. 1988. Cholecystokinin-58 is the major molecular form in man, dog and cat but not in pig, beef and rat intestine. Peptides 9: 993-998.

Edwards, C. M., S. Abusnana, D. Sunter, K. G. Murphy, M. A. Ghatei, and S. R. Bloom. 1999. The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. The Journal of Endocrinology 160: 7-12.

Efron, B. and R. J. Tibshirani. 1993. Standard errors and estimated standard errors & The Bootstrap estimate of standard errors. Chapman and Hall, NY.

Elliot, J. M. 1980. Propionate metabolism and vitamin B_{12}. Digestive Physiology and Metabolism in Ruminants. AVI Publishing Company, Westport, CT.

Elliot, J. M., H. W. Symonds, and B. Pike. 1985. Effect on Feed Intake of Infusing Sodium Propionate or Sodium Acetate into a Mesenteric Vein of Cattle. Journal of Dairy Science 68: 1165-1170.

Erdmann, J., F. Lippl, and V. Schusdziarra. 2003. Differential effect of protein and fat on plasma ghrelin levels in man. Regulatory Peptides 116: 101-107.

Erickson, J. C., K. E. Clegg, and R. D. Palmiter. 1996. Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. Nature 381: 415-421.

Fallingborg, J. 1999. Intra-luminal pH of the human gastrointestinal tract. Danish Medical Bulletin 46: 183-196.

Farningham, D. A., and C. C. Whyte. 1993. The role of propionate and acetate in the control of food intake in sheep. British Journal of Nutrition 70: 37-46.

Faulkner, A. and P. A. Martin. 1997. The concentrations of some gut polypeptides are elevated during lactation in ruminants. Comparative Biochemistry and Physiology 118: 563-568.

Faulkner, A., and H. T. Pollock. 1991. Effects of truncated glucagon-like peptide-1 on the responses of starved sheep to glucose. Journal of Endocrinology 129: 55-58.

Faverdin, P. 1999. The effect of nutrients on feed intake in ruminants. Proceedings of the Nutrition Society 58: 523-531.

Fehmann, H. C., and J. F. Habener. 1992. Insulinotropic hormone glucagon-like peptide-I(7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma beta TC-1 cells. Endocrinology 130: 159-166.

Fisher, G. E. J., A. M. Dowdeswell, and G. Perrott. 1996. The effects of sward characteristics and supplement type on the herbage intake and milk production of summer-calving cows. Grass and Forage Science 51: 121-130.

Flanagan, D. E., M. L. Evans, T. P. Monsod, F. Rife, R. A. Heptulla, W. V. Tamborlane, and R. S. Sherwin. 2003. The influence of insulin on circulating ghrelin. American journal of physiology. Endocrinology and Metabolism 284: 313-316.

Flemstrom, G. and A. Garner. 1982. Gastro-duodenal HCO_3 transport; characteristics and proposed role in acidity regulation and mucosal protection. American Journal of Physiology 242: 183-193.

Flier, J. S. 2004. Obesity wars: molecular progress confronts an expanding epidemic. Cell Death and Differentiation 116: 337-350.

Flint, A., A. Raben, A. Astrup, and J. J. Holst. 1998. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. Journal of Clinical Investigations 101: 515-520.

Forbes, J. M. 1988. Metabolic aspects of the regulation of voluntary food intake and appetite. Nutrition Research Reviews 1: 145-168.

Forbes, J. M. 1992. Metabolic aspects of satiety. Proceedings of the Nutrition Society 51: 13-19.

Forbes, J. M. 2007. Voluntary Food Intake and Diet Selection in farm Animals. 2nd edition. CAB Int, Wallingford, UK.

Forbes, S., S. Bui, B. R. Robinson, U. Hochgeschwender, and M. B. Brennan. 2001. Integrated control of appetite and fat metabolism by the leptin-proopiomelanocortin pathway. Proceedings of the National Academy of Sciences of the United States of America 98: 4233-4237.

Foster, L. A., N. K. Ames, and R. S. Emery. 1991. Food intake and serum insulin responses to intraventricular infusions of insulin and IGF-I. Physiology and Behaviour 50: 745-749.

Frandson, R. D., W. L. Wilke, and A. D. Fails. 2006. Anatomy and Physiology of Farm Animals. 6th Edition. Blackwell Publishing, Oxford, UK.

Furuse, M., S. I. Yang, Y. H. Choi, N. Kawamura, A. Takahashi, and J. Okomura. 1991. A note on plasma cholecystokinin concentration in dairy cows. Animal Production 53: 123-125.

Gao, S., K. P. Kinzig, S. Aja, K. A. Scott, W. Keung, S. Kelly, K. Strynadka, S. Chohnan, W. W. Smith, K. L. Tamashiro, E. E. Ladenheim, G. V. Ronnett, Y. Tu, M. J. Birnbaum, G. D. Lopaschuk, and T. H. Moran. 2007. Leptin activates hypothalamic acetyl-CoA carboxylase to inhibit food intake. Proceedings of the National Academy of Sciences of the United States of America 104: 17358-17363.

Gao, S., and M. D. Lane. 2003. Effect of the anorectic fatty acid synthase inhibitor C75 on neuronal activity in the hypothalamus and brainstem. Proceedings of the National Academy of Sciences of the United States of America 100: 5628-5633.

Garton, G. A., A. K. Lough, and E. Vioque. 1961. Glyceride hydrolysis and glycerol fermentation by sheep rumen contents. Journal of Genetic Microbiology 25: 215-225

Gary, L. A., G. W. Sherritt, and E. B. Hale. 1970. Behaviour of charolais cattle on pasture. Journal of Animal Science 30: 203-206.

Gautvik, K. M., L. de Lecea, V. T. Gautvik, P. E. Danielson, P. Tranque, A. Dopazo, F. E. Bloom, and J. G. Sutcliffe. 1996. Overview of the most prevalent hypothalamus-specific mRNAs, as identified by directional tag PCR subtraction. Proceedings of the National Academy of Sciences of the United States of America 93: 8733-8738.

Ge, H., L. Huang, T. Pourbahrami, and C. Li. 2002. Generation of soluble leptin receptor by ectodomain shedding of membrane-spanning receptors in vitro and in vivo. Journal of Biological Chemistry 277: 45898-45903.

Geary, N. 1990. Pancreatic glucagon signals postprandial satiety. Neuroscience and Biobehavioural Reviews 14: 323-338.

Geary, N. 1998. Glucagon and the control of meal size. Satiation. From gut to brain. Oxford University Press, New York

Gekara, J., E. C. Prigge, W. B. Bryan, M. Schettini, E. L. Nestor, and E. C. Townsend. 2001. Influence of pasture sward height and concentrate supplementation on intake, digestibility and grazing time of lactating beef cows. Journal of Animal Science 79: 745-752.

Gibb, M. J. 2006. Grassland management with emphasis on grazing behaviour. In: Fresh Herbage for Dairy Cattle. A. Elgersma, J. Dijkstra and S. Tamminga. Springer. The Netherlands.

Gibb, M. J., C. A. Huckle, and R. Nuthall. 1998. Effect of time of day on grazing behaviour by lactating dairy cows. Grass and Forage Science 53: 41-46.

Gibb, M. J., C. A. Huckle, R. Nuthall, and A. J. Rook. 1997. Effect of sward surface height on intake and grazing behaviour by lactating Holstein Friesian cows. Grass and Forage Science 52: 309-321.

Gibbs, J., R. C. Young, and G. P. Smith. 1973. Cholecystokinin elicits satiety in rats with open gastric fistulas. Nature 245: 323-325.

Gluckman, P. D., J. J. Johnson-Barrett, J. H. Butler, B. W. Edgar, and T. R. Gunn. 1983. Studies of insulin-like growth factor -I and -II by specific radioligand assays in umbilical cord blood. Clinical Endocrinology 19: 405-413.

Gomez-Ambrosi, J., V. Catalan, and G. Fruhbeck. 2009. The adipo-hepato-insular axis in glucose homeostasis. Peptides in Energy Balance and Obesity. CABI Int., Oxfordshire, UK. pp 398.

Gomez-Ambrosi, J. and G. Fruhbeck. 2005. Resistin: a promising therapeutic target for the management of type 2 diabetes mellitus? Drug Design Reviews 2: 1-12.

Gordon, J. G. 1968. Rumination and its significance. World Review of Nutrition and Dietetics 9: 251-273.

Graham, M., J. R. Shutter, U. Sarmiento, I. Sarosi, and K. L. Stark. 1997. Overexpression of AGRT leads to obesity in transgenic mice. Nature Genetics 17: 273-274.

Grandt, D., M. Schimiczek, C. Beglinger, P. Layer, H. Goebell, V. E. Eysselein, and J. R. Reeve, Jr. 1994. Two molecular forms of peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY 1-36 and PYY 3-36. Regulatory Peptides 51: 151-159.

Grandt, D., M. Schimiczek, W. Rascher, F. Feth, J. Shively, T. D. Lee, M. T. Davis, J. R. Reeve, Jr., and M. C. Michel. 1996. Neuropeptide Y 3-36 is an endogenous ligand selective for Y2 receptors. Regulatory Peptides 67: 33-37.

Grant, R. J. and J. L. Albright. 1995a. Feeding behaviour and management factors during the transition period in dairy cattle. Journal of Animal Science 73: 2791-2803.

Green, J. D. and G. W. Harris. 1949. Observation of the hypophysio-portal vessels of the living rat. The Journal of Physiology 108: 359-361.

Greenman, Y., N. Golani, S. Gilad, M. Yaron, R. Limor, and N. Stern. 2004. Ghrelin secretion is modulated in a nutrient- and gender-specific manner. Clinical Endocrinology 60: 382-388.

Gregorini, P., S. A. Gunter, and C. A. Masino. 2006. Daily grazing patterns of cattle: A behavioural overview. The Professional Animal Scientist 22: 201-209.

Gregorini, P., S. A. Gunter, C. A. Masino, and P. A. Beck. 2007. Effects of ruminal fill on short-term herbage intake rate and grazing dynamics of beef heifers. Grass and Forage Science 62: 346-354.

Grovum, W. L. 1981. Cholecystokinin administered intravenously did not act directly on the central nervous system or on the liver to supress food intake by sheep. British Journal of Nutrition 42: 183-201.

Gu, J., J. M. Polak, T. E. Adrian, J. M. Allen, K. Tatemoto, and S. R. Bloom. 1983. Neuropeptide tyrosine (NPY): a major cardiac neuropeptide. Lancet 1: 1008-1010.

Gutzwiller, J. P., B. Göke, J. Drewe, P. Hildebrand, S. Ketterer, D. Handschin, R. Winterhalder, D. Conen, and C. Beglinger. 1999. Glucagon-like peptide-1: a potent regulator of food intake in humans. Gut 44: 81-86.

Habel, R. E. 1975. Ruminant digestive system. The Anatomy of the Domestic Animals. W.B. Saunders Co., Philadelphia.

Hafez, E. S. E. 1969. The Behaviour of Domestic Animals. The Williams and Wilkins Company, Baltimore, USA.

Hagan, M. M., P. A. Rushing, S. C. Benoit, S. C. Woods, and R. J. Seeley. 2001. Opioid receptor involvement in the effect of AgRP- (83-132) on food intake and food selection. American Journal of Physiology, Regulatory, Integrative and Comparative Physiology 280: 814-821.

Hagan, M. M., P. A. Rushing, L. M. Pritchard, M. W. Schwartz, A. M. Strack, L. H. Van Der Ploeg, S. C. Woods, and R. J. Seeley. 2000. Long-term orexigenic effects of AgRP-(83-132) involve mechanisms other than melanocortin receptor blockade. American Journal of Physiology, Regulatory, Integrative and Comparative Physiology 279: 47-52.

Hahn, T. M., J. F. Breininger, D. G. Baskin, and M. W. Schwartz. 1998. Co-expression of AgRP and NPY in fasting-activated hypothalamic neurons. Nature Neuroscience 1: 271-272.

Hales, C. N., and P. J. Randle. 1963. Immunoassay of insulin with insulin-antibody precipitate. Journal of Biochemistry 88: 137-130.

Hamilton, K. C. 2009. Robust Estimates of Variance. Brookes and Cole, CA.

Hanson, R. W. and F. J. Ballard. 1967. The relative significance of acetate and glucose as precursors for lipid synthesis in liver and adipose tissue from ruminants. Journal of Biochemistry 105: 529-536.

Harding, R. and B. F. Leek. 1973. Central projections of gastric afferent vagal inputs. The Journal of Physiology 228: 73-90.

Harfoot, C. G. and G. P. Hazelwood. 1988. Lipid metabolism in the rumen. In: The Rumen Microbial Ecosystem. Elsevier Applied Science, London, U.K., pp 285-322.

Harmeyer, J. and U. Kollenkirchen. 1989. Thaimin and niacin in ruminant nutrition. Nutrition Research Reviews 21: 201-225.

Harmon, D. L. 1992. Impact of nutrition on pancreatic exocrine and endocrine secretion in ruminants: A review. Journal of Animal Science 70: 1290-1301.

Harris, B. L., J. M. Clark, and R. G. Jackson. 1996. Across-breed evaluation of dairy cattle. Proceedings of New Zealand Animal Production Society 56: 12-15.

Harris, B. L. and E. S. Kolver. 2001. Review of holsteinization of intensive pastoral dairy farming in New Zealand Journal of Dairy Science 84: 56-61.

Harrison, J., P. Findlay, D. Miller, and C. Adam. 2003. Intracerebroventricular ghrelin injection acutely stimulates food intake and inhibits luteinising hormone secretion in sheep. In: Endocrine Abstracts. pp 179.

Hastings, E. G. 1944. The significance of the bacteria and the protozoa of the rumen of the bovine. Bacteriology Reviews 8: 235-254.

Hauner, H. 2009. Elements of the adipostat. Peptides in Energy Balance and Obesity. CABI Oxfordshire, UK, pp 115-132.

Hayashida, T., K. Murakami, K. Mogi, M. Nishihara, M. Nakazato, M. S. Mondal, Y. Horii, M. Kojima, K. Kangawa, and N. Murakami. 2001. Ghrelin in domestic animals: distribution in stomach and its possible role. Domestic Animal Endocrinology 21: 17-24.

Heitmann, R. N., D. J. Dawes, and S. C. Sensenig. 1987. Hepatic ketogenesis and peripheral ketone body utilization in the ruminant. The Journal of Nutrition 117: 1174-1180.

Herath, C., G. Reynolds, D. MacKenzie, S. Davis, and P. Harris. 1999. Vagotomy suppresses cephalic phase insulin release in sheep. Experimental Physiology 84: 559-569.

Hetherington, A. W. and S. W. Ransob. 1940. Hypothalamic lesions and adiposity in the rat. Anatomical Records 78: 149-172.

Higgs, R. J., A. J. Sheahan, K. S. Mandok, M. E. Van Amburgh, and J. R. Roche. 2013. The effect of starch-, fibre-, or sugar-based supplements on nitrogen utilization in grazing dairy cows. Journal of Dairy Science 96: 3857-3866

Hillebrand, J. J., D. de Wied, and R. A. Adan. 2002a. Neuropeptides, food intake and body weight regulation: a hypothalamic focus. Peptides 23: 2283-2306.

Hodgson, J., and I. M. Brooks. 1999. Nutrition of Grazing Animals. New Zealand Pasture and Crop Science. Oxford University Press, Auckland.

Holmes, C. W., I. M. Brookes, D. J. Garrick, D. D. S. Mackensie, T. J. Parkinson, and G. F. Wilson. 2003. Milk Production from Pasture. Massey University, Palmerston North, New Zealand.

Holz, G. G. t., W. M. Kuhtreiber, and J. F. Habener. 1993. Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1 (7-37). Nature 361: 362-365.

Hoover, W. H. 1986. Chemical factors involved in ruminal fibre digestion. Journal of Dairy Science 69: 2755-2766.

Hoover, W. H. and S. R. Stokes. 1991. Balancing carbohydrates and proteins for optimum rumen microbial yield. Journal of Dairy Science 74: 3630-3644.

Horan, B., P. Dillon, P. Faverdin, L. Delaby, F. Buckley, and M. Rath. 2005. The interaction of strain of Holstein-Friesian cows and pasture-based feed systems on milk yield, body weight and body condition score. Journal of Dairy Science 88: 1231-1243.

Horan, B., P. Faverdin, L. Delaby, F. Buckley, and M. Rath. 2006. The effect of strain of holstein-friesian dairy cow and pasture-based system on grass intake and milk production. Journal of Animal Science 82: 435-444.

Horan, B., J. F. Mee, M. Rath, P. O'Connor, and P. Dillon. 2004. The effect of strain of holstein-friesian cow and feeding systems on reproductive performance in seasonal-calving milk production systems. Journal of Animal Science 79: 453-467.

Horino, M., L. J. Machlin, F. Hertelendy, and D. M. Kipnis. 1968. Effect of short-chain fatty acids on plasma insulin in ruminant and nonruminant species. Endocrinology 83: 118-128.

Horvath, T. L., I. Bechmann, F. Naftolin, S. P. Kalra, and C. Leranth. 1997. Heterogeneity in the neuropeptide Y-containing neurons of the rat arcuate nucleus: GABAergic and non-GABAergic subpopulations. Brain Research 756: 283-286.

Horvath, T. L., S. Diano, P. Sotonyi, M. Heiman, and M. Tschop. 2001. Mini review: ghrelin and the regulation of energy balance. A hypothalamic perspective. Endocrinology 142: 4163-4169.

Hove, K., and A. K. Blom. 1973. Plasma insulin and growth hormone in dairy cows; diurnal variation and relation to food intake and plasma sugar and acetoacetate levels. Acta Endocrinologica 73: 289-303.

Hug, C., J. Wang, N. S. Ahmad, J. S. Bogan, T. S. Tsao, and H. F. Lodish. 2004. T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. Proceedings of the National Academy of Sciences of the United States of America 101: 10308-10313.

Hume, I. D. 1999. Marsupial Nutrition. Cambridge University Press. Cambridge, UK.

Hungate, R. E. 1966. The Rumen and its Microbes. Academic Press, New York.

Huntington, G. B. 1986. Uptake and transport of non-protein nitrogen by the ruminant gut. Federation Proceedings 45: 2272-2276.

Huntington, G. B., D. L. Harmon, and C. J. Richards. 2006. Sites, rates and limits of starch digestion and glucose metabolism in growing cattle. Journal of Animal Science 84: 14-24.

Hussain, M. A., P. B. Daniel, and J. F. Habener. 2000. Glucagon stimulates expression of the inducible cAMP early repressor and suppresses insulin gene expression in pancreatic beta-cells. Diabetes 49: 1681-1690.

Iggo, A. 1954. Receptors in the stomach and the bladder. The Journal of Physiology 126: 29-30.

Iggo, A. 1955. Tension receptors in the stomach and the urinary bladder. The Journal of Physiology 128: 593-607.

Ikwuegbu, O. A. and J. D. Sutton. 1982. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. British Journal of Nutrition 48: 365.

Illius, A. W. and N. S. Jessop. 1996. Metabolic constraints on voluntary intake in ruminants. Journal of Animal Science 74: 3052-3062.

Irwin, D. M., and A. C. Wilson. 1990. Concerted evolution of ruminant stomach lysozymes. The Journal of Biological Chemistry 265: 4944-4952.

Ishler, V., J. Heinrichs, and G. Varga. 1996. From feed to milk: Understanding rumen function. Pennsylvania State University, Pennsylvania.

Jami, L. 1992. Golgi tendon organs in mammalian skeletal muscle: functional properties and central actions. Physiological Reviews 72: 623-666.

Janis, C. 1976. The evolutionary strategy of the equidae and the origins of rumen and cecal digestion. Evolution 30: 757-774.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. Journal of Dairy Science 76: 3851-3863.

Jequier, E. 2002. Leptin signalling, adiposity, and energy balance. Annals of the New York Academy of Sciences 967: 379-388.

Jermann, T. M., J. G. Opitz, J. Stackhouse, and S. A. Benner. 1995. Reconstructing the evolutionary history of the artiodactyl ribonuclease superfamily. Nature 374: 57-59.

Jiang, G. and B. B. Zhang. 2003. Glucagon and regulation of glucose metabolism. American journal of physiology. Endocrinology and Metabolism 284: 671-678.

Jolles, P. and J. Jolles. 1984. What's new in lysozyme research? Molecular and Cellular Biochemistry 63: 165-189.

Jolles, P., F. Schoentgen, J. Jolles, D. E. Dobson, E. M. Prager, and A. C. Wilson. 1984. Stomach lysozymes of ruminants. II. Amino acid sequence of cow lysozyme 2 and immunological comparisons with other lysozymes. Journal of Biological Chemistry 259: 11617-11625.

Jouany, J. P., B. Michealet-Doreau, and M. Doreau. 1998. Manipulation of the rumen Ecosystem to support high-performance beef cattle: Review. In: The 8th World Conference on Animal Production, Seoul, Korea

Kadowaki, T. and T. Yamauchi. 2005. Adiponectin and adiponectin receptors. Endocrine Reviews 26: 439-451.

Kastin, A. J. and W. Pan. 2000. Dynamic regulation of leptin entry into brain by the blood-brain barrier. Regulatory Peptides 92: 37-43.

Kennedy, E., M. O'Donovan, L. Delaby, and F. P. O'Mara. 2008. Effect of herbage allowance and concentrate supplementation on dry matter, milk production and energy balance of early lactating dairy cows. Livestock Science 117: 275–286.

Kennedy, G. C. 1953. The role of depot fat in the hypothalamic control of food intake in the rat. Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character. Royal Society 140: 578-596.

Kennedy, J., P. Dillon, L. Delaby, P. Faverdin, G. Stakelum, and M. Rath. 2003. Effect of genetic merit and concentrate supplementation on grass intake and milk production with Holstein-Friesian dairy cows. Journal of Dairy Science 86: 610-621.

Kershaw, E. E. and J. S. Flier. 2004. Adipose tissue as an endocrine organ. he Journal of Clinical Endocrinology and Metabolism 89: 2548-2556.

Kertz, A. F., L. F. Reutzel, and G. M. Thomson. 1991. Dry matter intake from parturition to mid lactation. Journal of Dairy Science 74: 2290-2295.

Kieffer, T. J. and J. F. Habener. 1999. The glucagon-like peptides. Endocrinology Reviews 20: 876-913.

Kieffer, T. J. and J. F. Habener. 2000. The adipoinsular axis: effects of leptin on pancreatic beta-cells. American Journal of Physiology. Endocrinology and Metabolism 278: 1-14.

Kieffer, T. J., R. S. Heller, C. G. Unson, G. C. Weir, and J. F. Habener. 1996. Distribution of glucagon receptors on hormone-specific endocrine cells of rat pancreatic islets. Endocrinology 137: 5119-5125.

Kieffer, T. J., C. H. McIntosh, and R. A. Pederson. 1995. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. Endocrinology 136: 3585-3596.

Kil, S. J. and M. A. Froetschel. 1994. Involvement of opiod peptides from casein on reticular motility and digesta passage in steers. Journal of Dairy Science 77: 111-123.

Kim, E. J., S. A. Huws, M. R. F. Lee, and N. D. Scollan. 2009. Dietary transformation of lipid in the rumen microbial ecosystem. Asian-Australian Journal of Animal Science 22: 1341-1350.

Kim, E. M., M. K. Grace, C. C. Welch, C. J. Billington, and A. S. Levine. 1999. STZ-induced diabetes decreases and insulin normalizes POMC mRNA in arcuate nucleus and pituitary in rats. The American Journal of Physiology 276: 1320-1326.

Kissileff, H. R., F. X. Pi-Sunyer, J. Thornton, and G. P. Smith. 1981. C-terminal octapeptide of cholecystokinin decreases food intake in man. American Journal of Clinical Nutrition 34: 154-160.

Knapp, J. R., H. C. Freetly, B. L. Reis, C. C. Calvert, and R. L. Baldwin. 1992. Effects of somatotropin and substrates on patterns of liver metabolism in lactating dairy cattle. Journal of Dairy Science 75: 1025-1035.

Kojima, M., H. Hosoda, Y. Date, M. Nakazato, H. Matsuo, and K. Kangawa. 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402: 656-660.

Kojima, M., H. Hosoda, H. Kaiya, and K. Kangawa. 2004. Discovery of ghrelin, an endogenous ligand for the growth-hormone secretagogue receptor. In: Ghrelin. Kluwer Academic Publishers, USA, pp 27-45.

Kojima, M. and K. Kangawa. 2005. Ghrelin: structure and function. Physiological Reviews 85: 495-522.

Kolaczynski, J. W., M. R. Nyce, R. V. Considine, G. Boden, J. J. Nolan, R. Henry, S. R. Mudaliar, J. Olefsky, and J. F. Caro. 1996. Acute and chronic effects of insulin on leptin production in humans: Studies in vivo and in vitro. Diabetes 45: 699-701.

Kolver, E. S. and M. J. de Veth. 2002. Prediction of ruminal pH from pasture-based diets. Journal of Dairy Science 85: 1255-1266.

Kolver, E. S. and L. D. Muller. 1998. Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. Journal of Dairy Science 81: 1403-1411.

Kolver, E. S., J. R. Roche, M. J. de Veth, and A. R. Napper. 2002. Total mixed rations verses pasture diets; Evidence for a genotype x diet interaction in dairy cow performance. In: Proceedings of the New Zealand Society of Animal Production. pp 246-251.

Komatsu, T., F. Itoh, S. Mikawa, and K. Hodate. 2003. Gene expression of resistin in adipose tissue and mammary gland of lactating and non-lactating cows. The Journal of Endocrinology 178: 1-5.

Konturek, S. J., J. W. Konturek, T. Pawlik, and T. Brzozowski. 2004. Brain-gut axis and its role in the control of food intake. Journal of Physiology and Pharmacology : An Official Journal of the Polish Physiological Society 55: 137-154.

Koopmans, S. J., M. Frolich, E. H. Gribnau, R. G. Westendorp, and R. A. DeFronzo. 1998. Effect of hyperinsulinemia on plasma leptin concentrations and food intake in rats. The American Journal of Physiology 274: 998-1001.

Kornegay, J. R., J. W. Schilling, and A. C. Wilson. 1994. Molecular adaptation of a leaf-eating bird: Stomach lysozyme of the Hoatzin. Molecular Biology and Evolution 11: 921-928.

Krause, D. O., B. P. Dalrymple, W. J. Smith, R. I. Mackie, and C. S. McSweeney. 1999. 16S rDNA sequencing of *Ruminococcus albus* and *Ruminococcus flavefaciens*: design of a signature probe and its application in adult sheep. Microbiology 145: 1797-1807.

Krehbiel, C. R., D. L. Harmon, and J. E. Schneider. 1992. Effect of increasing ruminal butyrate on portal and hepatic nutrient flux in steers. Journal of Animal Science 70: 904-914.

Kristensen, N. B. 2001. Rumen microbial sequestration of acetate in cattle. Journal of Animal Science 79: 2491-2175.

Kristensen, N. B. 2005. Splanchnic metabolism of volatile fatty acids in the dairy cow. Journal of Animal Science 80: 3-10.

Kristensen, N. B. and D. L. Harmon. 2004. Effect of increasing ruminal butyrate absorption on splanchnic metabolism of volatile fatty acids absorbed from the washed reticulorumen of steers. Journal of Animal Science 82: 3549-3559.

Kristensen, N. B., S. G. Pierzynowski, and A. Danfaer. 2000. Net portal appearance of volatile fatty acids in sheep intraruminally infused with mixtures of acetate, propionate, isobutyrate, butyrate and valerate. Journal of Animal Science 78: 1372-1379.

Kristensen, P., M. E. Judge, L. Thim, U. Ribel, K. N. Christjansen, B. S. Wulff, J. T. Clausen, P. B. Jensen, O. D. Madsen, N. Vrang, P. J. Larsen, and S. Hastrup. 1998. Hypothalamic CART is a new anorectic peptide regulated by leptin. Nature 393: 72-76.

Krysl, L. J. and B. W. Hess. 1993. Influence of supplementation on behaviour of grazing cattle. Journal of Animal Science 71: 2546-2555.

Kuhar, M. J. and S. E. Dall Vechia. 1999. CART peptides: novel addiction and feeding-related neuropeptides. Trends in Neurosciences 22: 316-320.

Kulkarni, R. N., Z. L. Wang, R. M. Wang, J. D. Hurley, D. M. Smith, M. A. Ghatei, D. J. Withers, J. V. Gardiner, C. J. Bailey, and S. R. Bloom. 1997. Leptin rapidly suppresses insulin release from insulinoma cells, rat and human islets and, in vivo, in mice. Journal of Clinical Investigations 100: 2729-2736.

Lafontan, M., C. Sengenes, C. Moro, J. Galitzky, and M. Berlan. 2009. Natriuretic peptides and other lipolytic peptides involved in the control of lipid mobilization. Peptides in energy balance and Obesity. CAB Int., Oxfordshire, UK. pp 398.

Lambert, P. D., P. R. Couceyro, K. M. McGirr, S. E. Dall Vechia, Y. Smith, and M. J. Kuhar. 1998. CART peptides in the central control of feeding and interactions with neuropeptide Y. Synapse 29: 293-298.

Larsen, P. J., N. Vrang, P. C. Petersen, and P. Kristensen. 2000. Chronic intracerebroventricular administration of recombinant CART (42-89) peptide inhibits and causes weight loss in lean and obese Zucker (fa/fa) rats. Obesity Research 8: 590-596.

Layden, B. T., V. Durai, and J. Lowe, W.L. 2010. G-protein-coupled receptors, pancreatic islets and diabetes. Nature Education 3: 13.

Lee, G. H., R. Proenca, J. M. Montez, K. M. Carroll, J. G. Darvishzadeh, J. I. Lee, and J. M. Friedman. 1996. Abnormal splicing of the leptin receptor in diabetic mice. Nature 379: 632-635.

Lee, H. M., G. Wang, E. W. Englander, M. Kojima, and G. H. Greeley, Jr. 2002. Ghrelin, a new gastrointestinal endocrine peptide that stimulates insulin secretion: enteric distribution, ontogeny, influence of endocrine, and dietary manipulations. Endocrinology 143: 185-190.

Lee, S. H. and K. L. Hossner. 2002. Effects of propionate infusion on the expression of lipogenic genes and metabolic hormones in sheep. Animal Sciences Research Report, The Department of Animal Sciences, Colorado State University, Colorado State.

Leek, B. 1969. Reticulo-ruminal mechanoreceptors in sheep. Journal of Physiology 202: 585-609.

Leng, R. A. 1982. Dynamics of protozoa in the rumen of sheep. British Journal of Nutrition 48: 399-415.

Lents, C. A., R. P. Wettemann, F. J. White, I. Rubio, N. H. Ciccioli, L. J. Spicer, D. H. Keisler, and M. E. Payton. 2005. Influence of nutrient intake and body fat on concentrations of insulin-like growth factor-I, insulin, thyroxine, and leptin in plasma of gestating beef cows. Journal of Animal Science 83: 586-596.

Li, H. Y., H. W. Hwang, and Y. H. Hu. 2002. Functional characterizations of cocaine- and amphetamine-regulated transcript mRNA expression in rat hypothalamus. Neuroscience Letters 323: 203-206.

Li, J. Y., S. Finniss, Y. K. Yang, Q. Zeng, S. Y. Qu, G. Barsh, C. Dickinson, and I. Gantz. 2000. Agouti-related protein-like immunoreactivity: characterization of release from hypothalamic tissue and presence in serum. Endocrinology 141: 1942-1950.

Licinio, J., A. B. Negrao, C. Mantzoros, V. Kaklamani, M. L. Wong, P. B. Bongiorno, A. Mulla, L. Cearnal, J. D. Veldhuis, J. S. Flier, S. M. McCann, and P. W. Gold. 1998. Synchronicity of frequently sampled, 24-h concentrations of circulating leptin, luteinizing hormone, and estradiol in healthy women. Proceedings of the National Academy of Sciences of the United States of America 95: 2541-2546.

Liddle, R. A., I. D. Goldfine, M. S. Rosen, R. A. Taplitz, and J. A. Williams. 1985. Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction. Journal of Clinical Investigations 75: 1144-1152.

Liddle, R. A., E. T. Morita, C. K. Conrad, and J. A. Williams. 1986. Regulation of gastric emptying in humans by cholecystokinin. Journal of Clinical Investigations 77: 992-996.

Lieverse, R. J., J. B. Jansen, A. M. Masclee, and C. B. Lamers. 1994. Satiety effects of cholecystokinin in humans. Gastroenterology 106: 1451-1454.

Lin, H. C. and W. Y. Chey. 003. Cholecystokinin and peptide YY are released by fat in either proximal or distal small intestine in dogs. Regulatory Peptides 114: 131-135.

Linnane, M., B. Horan, J. M. Connolly, P. O'Connor, F. Buckley, and P. Dillon. 2004. The effect of strain of Holstein-Friesian and feeding system on grazing behaviour, herbage intake and productivity in the first lactation. Journal of Animal Science 78: 169-178.

Little, T. J., M. Horowitz, and C. Feinle-Bisset. 2005. Role of cholecystokinin in appetite control and body weight regulation. Obesity Reviews: An Official Journal of the International Association for the Study of Obesity 6: 297-306.

Loftus, T. M., D. E. Jaworsky, G. L. Frehywot, C. A. Townsend, G. V. Ronnett, M. D. Lane, and F. P. Kuhajda. 2000. Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. Science 288: 2379-2381.

Lopaschuk, G. D., J. R. Ussher, and J. S. Jaswal. 2010. Targeting intermediary metabolism in the hypothalamus as a mechanism to regulate appetite. Pharmacological Reviews 62: 237-264.

Lopez, M., L. Seoane, M. C. Garcia, F. Lago, F. F. Casanueva, R. Senaris, and C. Dieguez. 2000. Leptin regulation of prepro-orexin and orexin receptor mRNA levels in the hypothalamus. Biochemical and Biophysical Research Communications 269: 41-45.

López, M., S. Tovar, M. J. Vázquez, L. M. Williams, and C. Diégue. 2007. Peripheral tissue-brain interactions in the regulation of food intake. Proceedings of the Nutrition Society 66: 131-155.

Low, A. G. 1990. Nutritional regulation of gastric secretion, digestion and emptying. Nutrition Research Reviews: 229-252.

Lu, S., J. L. Guan, Q. P. Wang, K. Uehara, S. Yamada, N. Goto, Y. Date, M. Nakazato, M. Kojima, K. Kangawa, and S. Shioda. 2002. Immunocytochemical observation of ghrelin-containing neurons in the rat arcuate nucleus. Neuroscience Letters 321: 157-160.

Luginbuhl, J. 1983. Comparative Anatomy of the Digestive Tract in Cattle, Sheep and Goats. 40. North Carolina State University, Raleigh, NC.

Mackle, T. R., C. R. Parr, and A. M. Bryant. 1996. Nitrogen fertiliser effects on milk yield and composition pasture intake, nitrogen and energy partitioning, and rumen fermentation parameters of dairy cows in early lactation. New Zealand Journal of Agricultural Research 39: 341-356.

Maher, J., G. Stakelum, and M. Rath. 1997. The effect of level of daily herbage allowance on the performance of spring-calving dairy cows. In: Irish Agricultural Research Forum, Ireland. pp 217-218.

Maloiy, G. M. and E. T. Clemens. 1980. Gastrointestinal osmolality electrolyte and organic acid composition in five species of East African herbivorous mammals. Journal of Animal Science 51: 917-924.

Mangel, C. and C. W. Clark. 1986. Towards a unified foraging theory. Ecology 67: 1127-1138.

Manns, J. G., and J. M. Boda. 1967. Insulin release by acetate, propionate, butyrate and glucose in lambs and adult sheep. American Journal of Physiology 212: 747.

Margolis, R. U. and N. Altszuler. 1967. Insulin in the cerebrospinal fluid. Nature 215: 1375-1376.

Marquardt, J. P., R. L. Horst, and N. A. Jorgensen. 1977. Effect of parity on dry matter intake at parturition in dairy cattle 1. Journal of Dairy Science 60: 929-934.

Marsh, D. J., G. Hollopeter, K. E. Kafer, and R. D. Palmiter. 1998. Role of the Y5 neuropeptide Y receptor in feeding and obesity. Nature Medicine 4: 718-721.

Masson, M. J., and A. T. Phillipson. 1951. The absorption of acetate, propionate and butyrate from the rumen of sheep. Journal of Physiology 113: 189-206.

Mayne, C. S. 1991. Effects of supplementation on the performance of both growing and lactating cattle at pasture. Occasional Symposium of the British Grasslands Society 25: 55-71.

Mayne, C. S., and I. A. Wright. 1988. Herbage Intake and Utilization by the Grazing Dairy Cow. Butterworths, London.

McAllister, T. A. and K. J. Cheng. 1996. Microbial strategies in the ruminal digestion of cereal grains. Animal Feed Science and Technology 62: 29-36.

McCarthy, J. P., A. Faulkner, P. A. Martin, and D. J. Flint. 1992. Changes in the plasma concentration of gastric inhibitory polypeptide and other metabolites in response to feeding in sheep. The Journal of Endocrinology 134: 235-240.

McCarthy, S., D. P. Berry, P. Dillon, M. Rath, and B. Horan. 2007a. Influence of Holstein-Friesian strain and feed system on Body weight and body condition score lactation profiles. Journal of Dairy Science 90: 1859-1869.

McCarthy, S., B. Horan, M. Rath, M. Linnane, P. O'Connor, and P. Dillon. 2007b. The influence of strain of Holstein-Friesian dairy cow and pasture-based feeding system on grazing behaviour, intake and milk production. Grass and Forage Science 62: 13-26.

McGarry, J. D. and D. W. Foster. 1980. Regulation of hepatic fatty acid oxidation and ketone body production. Annual Reviews in Biochemistry 49: 753-766.

McGilloway, D. A. and C. S. Mayne. 1996. The importance of grass availability for the high genetic dairy cow. In: Recent Advances in Animal Nutrition. Nottingham University Press, Nottingham, pp 135-167.

McGowan, M. K., K. M. Andrews, and S. P. Grossman. 1992. Chronic intrahypothalamic infusions of insulin or insulin antibodies alter body weight and food intake in the rat. Physiology and Behaviour 51: 753-766.

McNamara, S., J. J. Murphy, M. Rath, and F. P. O'Mara. 2003. Effects of different transition diets on energy balance, blood metabolites and reproductive performance in dairy cows. Livestock Production Science 84: 195-206.

McTernan, P. G., C. M. Kusminski, and S. Kumar. 2006. Resistin. Current Opinion in Lipidology 17: 170-175.

Meier, J. J., B. Gallwitz, S. Salmen, O. Goetze, J. J. Holst, W. E. Schmidt, and M. A. Nauck. 2003. Normalization of glucose concentrations and deceleration of gastric emptying after solid meals during intravenous glucagon-like peptide 1 in patients with type 2 diabetes. The Journal of Clinical Endocrinology and Metabolism 88: 2719-2725.

Meijs, J. A. C. 1986. Concentrate supplementation of grazing dairy cows 2. Effect of concentrate composition on herbage intake and milk production. Grass and Forage Science 41: 229-235.

Mentlein, R., B. Gallwitz, and W. E. Schmidt. 1993. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1 (7-36) amide, peptide histidine methionine and is responsible for their degradation in human serum. European Journal of Biochemistry 214: 829-835.

Mercer, J. G., N. Hoggard, L. M. Williams, C. B. Lawrence, L. T. Hannah, P. J. Morgan, and P. Trayhurn. 1996. Coexpression of leptin receptor and preproneuropeptide Y mRNA in arcuate nucleus of mouse hypothalamus. Journal of Neuroendocrinology 8: 733-735.

Merchen, N. R. 1988. Digestion, absorption and excretion in ruminants. The Ruminant Animal Digestive Physiology and Nutrition. Reston Books, Englewoods Cliffs, pp 172-201.

Mertens, D. R. 1987. Predicting intake and digestibility using mathematical models of ruminal function. Journal of Dairy Science 64: 1548-1558.

Mertens, D. R. 1994. Regulation of forage intake. In: Forage Quality, Evaluation and Utilization. G.C. Fahey. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America pp 450-493.

Métais, G. and I. Vislobokova. 2007. Basal ruminants. In: The Evolution of the Artiodactyls. D.R. Prothero and S. E. Foss. pp 189-212. The John Hopkins University Press, Baltimore MD.

Metz, J. H. M. 1975. Time patterns of feeding and rumination in domestic cattle. PhD, Thesis. University of Wageningen. The Netherlands.

Miller, L. A., J. M. Moorby, D. R. Davies, M. O. Humphreys, N. D. Scollan, J. C. MacRae, and M. K. Theodorou. 2001. Increased concentration of water-soluble carbohydrate in perennial ryegrass *(Lolium perenne L.)*: milk production from late-lactation dairy cows. Grass and Forage Science 56: 383-394.

Miner, J. L., M. A. Della-Fera, J. A. Paterson, and C. A. Baile. 1989. Lateral cerebroventricular injection of neuropeptide Y stimulates feeding in sheep. The American Journal of Physiology 257: 383-387.

Minokoshi, Y., T. Alquier, N. Furukawa, Y. B. Kim, A. Lee, B. Xue, J. Mu, F. Foufelle, P. Ferre, M. J. Birnbaum, B. J. Stuck, and B. B. Kahn. 2004. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature 428: 569-574.

Minor, S., N. A. Macleod, T. R. Preston, and R. A. Leng. 1977. Studies on digestion in different sections of the intestinal tract of bulls fed sugar cane/urea with different supplements. Tropical Animal Production 2: 163-174.

Moran, J. 2005a. How Feed Requirements Change During Lactation. Landlinks Press, Collingwood, Australia.

Moran, J. 2005b. How the Rumen Works. Landlinks Press, Collingwood, Australia.

Moran, T. H. and K. P. Kinzig. 2004. Gastrointestinal satiety signals II. Cholecystokinin. American Journal of Physiology. Gastrointestinal and Liver Physiology 286: 183-188.

Morrison, C. D., J. A. Daniel, B. J. Holmberg, J. Djiane, N. Raver, A. Gertler, and D. H. Keisler. 2001. Central infusion of leptin into well-fed and undernourished ewe lambs: effects on feed intake and serum concentrations of growth hormone and luteinizing hormone. The Journal of Endocrinology 168: 317-324.

Morton, G. J., D. E. Cummings, D. G. Baskin, G. S. Barsh, and M. W. Schwartz. 2006. Central nervous system control of food intake and body weight. Nature 443: 289-295.

Moss, A. R., J. Jouany, and J. Newbold. 2000. Methane production by ruminants: its contribution to global warming. Annales De Zootechnie 49: 251-253.

Mould, F. L., E. R. Ørskov, and S. O. Mann. 1983. Associative effects of mixed feeds. I. effects of type and level of supplementation and the influence of the rumen fluid pH on cellulolysis in vivo and dry matter digestion of various roughages. Animal Feed Science and Technology 10: 15-30.

Murakami, N., T. Hayashida, T. Kuroiwa, K. Nakahara, T. Ida, M. S. Mondal, M. Nakazato, M. Kojima, and K. Kangawa. 2002. Role for central ghrelin in food intake and secretion profile of stomach ghrelin in rats. The Journal of Endocrinology 174: 283-288.

Murdolo, G., P. Lucidi, C. Di Loreto, N. Parlanti, A. De Cicco, C. Fatone, C. G. Fanelli, G. B. Bolli, F. Santeusanio, and P. De Feo. 2003. Insulin is required for prandial ghrelin suppression in humans. Diabetes 52: 2923-2927.

Murphy, K. G. and S. R. Bloom. 2006. Gut hormones and the regulation of energy homeostasis. Nature 444: 854-859.

Murphy, K. G., W. S. Dhillo, and S. R. Bloom. 2006. Gut peptides in the regulation of food intake and energy homeostasis. Endocrine Reviews 27: 719-727.

Murphy, M. R., R. L. Baldwin, and L. J. Koong. 1982. Estimation of stoichiometric parameters for rumen fermentation of roughage and concentrate diets. Journal of Animal Science 55: 411-421.

Muurahainen, N., H. R. Kissileff, A. J. Derogatis, and F. X. Pi-Sunyer. 1988. Effects of cholecystokinin-octapeptide (CCK-8) on food intake and gastric emptying in man. Physiology and Behaviour 44: 645-649.

Myers, M. J. 2004. Leptin receptor signalling and the regulation of mammalian physiology. Recent Progress in Hormone Research 59: 287-304.

Nakai, Y., H. Hosoda, K. Nin, C. Ooya, H. Hayashi, T. Akamizu, and K. Kangawa. 2003. Plasma levels of active form of ghrelin during oral glucose tolerance test in patients with anorexia nervosa. European Journal Endocrinology 149: 1-3.

Neary, N. M., A. P. Goldstone, and S. R. Bloom. 2004. Appetite regulation: from the gut to the hypothalamus. Clinical Endocrinology 60: 153-160.

Nicholson, T., and S. A. Omer. 1983. The inhibitory effect of intestinal infusions of unsaturated long-chain fatty acids on forestomach motility of sheep. British Journal of Nutrition 50: 141-149.

Niijima, A. 1969. Afferent impulse discharges from glucoreceptors in the liver of the guinea pig. Annals of the New York Academy of Sciences 157: 690-700.

NRC. 2001. Nutritional Requirements of Dairy Cattle. 7th National Academy of Science, Washington, DC.

O'Connell, J. M., F. Buckley, M. Rath, and P. Dillon. 2000. The effects of cow genetic merit and feeding treatment on milk production, herbage intake and grazing behaviour of dairy cows. Irish Journal of Agricultural and Food Research 39: 369-381.

Oba, M. and M. S. Allen. 2003. Dose-response effects of intra-ruminal infusion of propionate on feeding behaviour of lactating cows in early or mid lactation. Journal of Dairy Science. 86: 2922-2931.

Oetjen, E., T. Diedrich, A. Eggers, B. Eckert, and W. Knepel. 1994. Distinct properties of the cAMP-responsive element of the rat insulin I gene. Journal of Biological Chemistry 269: 27036-27044.

Olsson, G. M., M. Emmanualson, and H. Wiktorsson. 1998. Effects of different nutritional levels prepartum on the subsequent performance of dairy cows. Livestock Production. Science 18: 1-17.

Onaga, T., M. Yoshida, H. Inoue, and H. Yokota. 2000. Regional distribution and plasma concentration of peptide YY in sheep. Peptides 21: 655-667.

Orpin, C. G. 1983. The role of ciliate protozoa and fungi in the rumen digestion of plant cell walls. Animal Feed Science and Technology 10: 121-143.

Orr, R. J., S. M. Rutter, P. D. Penning, and A. J. Rook. 2001. Matching grass supply to grazing patterns for dairy cows. Grass and Forage Science 56: 352-361.

Orskov, C., A. Wettergren, and J. J. Holst. 1993. Biological effects and metabolic rates of glucagon like peptide-1 7-36 amide and glucagon like peptide-1 7-37 in healthy subjects are indistinguishable. Diabetes 42: 658-661.

Orskov, E. R. 1972. Reflex closure of the oesophageal groove and its potential application in ruminant nutrition. South African Journal of Animal Science 2: 169-176.

Orskov, E. R. 1986. Starch digestion and utilization in ruminants. Journal of Animal Science 63: 1624-1633.

Ørskov, E. R. 1987. Feed intake. In: The Feeding of Ruminants: Principles and Practice. 2nd ed. Chalcombe.

Orskov, E. R. and C. Fraser. 1975. The effects of processing of barley-based supplements on rumen pH, rate of digestion and voluntary intake of dried grass in sheep. British Journal of Nutrition 34: 493-500.

Overduin, J., R. S. Frayo, H. J. Grill, J. M. Kaplan, and D. E. Cummings. 2005. Role of the duodenum and macronutrient type in ghrelin regulation. Endocrinology 146: 845-850.

Pandya, P. K., S. C. Huang, V. D. Talkad, S. A. Wank, and J. D. Gardner. 1994. Biochemical regulation of the three different states of the cholecystokinin (CCK) receptor in pancreatic acini. Biochimica et Biophysica Acta 1224: 117-126.

Pape, J. R. and G. Tramu. 1996. Suckling-induced changes in neuropeptide Y and proopiomelanocortin gene expression in the arcuate nucleus of the rat: evaluation of a putative intervention of prolactin. Neuroendocrinology 63: 540-549.

Parker, W. J. and S. N. McCutcheon. 1992. Effect of sward height on herbage intake and production of ewes of different rearing rank during lactation. The Journal of Agricultural Science 118: 383-395.

Pathak, A. K. 2008. Various factors affecting microbial protein synthesis in the rumen. Veterinary World 1: 186-189.

Patterson, D. M., F. J. Gordon, C. S. Mayne, M. G. Porter, and E. F. Unsworth. 1995. The effect of genetic merit in nutrient utillisation in lactating dairy cows. British Society of Animal Production, Occasional Publication No. 19.

Pavlov, I. P. 1902. The work of the digestive glands. Charles Griffin Co Ltd, London.

Pearce, G. R., and R. J. Moir. 1964. Rumination in sheep I. The influence of rumination and grinding upon the passage and digestion of food. Australian Journal of Agricultural Research 15: 635-644.

Pedrazzini, T., J. Seydoux, P. Kunstner, J. F. Aubert, E. Grouzmann, F. Beermann, and H. R. Brunner. 1998. Cardiovascular response, feeding behaviour and locomotor activity in mice lacking the NPY Y1 receptor. Nature Medicine 4: 722-726.

Penning, P. D., A. J. Rook, and R. J. Orr. 1991. Patterns of ingestive behaviour of sheep continuously stocked on monocultures of ryegrass or white clover. Applied Animal Behavioural Science 31: 237-250.

Pennington, R. J. 1952. The metabolism of short -chain fatty acids in the sheep 1. Fatty acid utilization and ketone body production by rumen epithelium and other tissues. Journal of Biochemistry 51: 251-258.

Penno, J. W. 2002. The response by grazing dairy cows to supplementary feeds. PhD, Massey University, New Zealand.

Perboni, S., N. Ueno, G. Mantovani, and A. Inui. 2009. Anorexigenic peptides. Peptides in Energy Balance and Obesity. CAB Int, Oxfordshire, U.K., pp 396.

Perez-Prieto, L. A., J. L. Peyrayd, and R. Delagarde. 2011. Substitution rate and milk yield response to corn silage supplementation of late-lactation dairy cows grazing low-mass pastures at 2 daily allowances in autumn. Journal of Dairy Science 94: 3592-3604.

Peruzzo, B., F. E. Pastor, J. L. Blazquez, K. Schobitz, B. Pelaez, P. Amat, and E. Rodriguez. 2000. A Second look at the Barriers of the Medial basal Hypothalamus. Experimental Brain Research. Experimentelle Hirnforschung. Experimentation Cerebrale 132: 10-26.

Philippe, J. and M. Missotten. 1990. Functional characterization of a cAMP-responsive element of the rat insulin I gene. Journal of Biological Chemistry 265: 1465-1469.

Phillips, C. J. C. and J. D. Leaver. 1986. The effect of forage supplementation on the behaviour of grazing dairy cows. Applied Animal Behaviour Science 16: 233-247.

Phillips, R. J. and T. L. Powley. 2000. Tension and stretch receptors in gastrointestinal smooth muscle: re-evaluating vagal mechanoreceptor electrophysiology. Brain Research Reviews 34: 1-26.

Pierroz, D. D., M. Ziotopoulou, L. Ungsunan, S. Moschos, J. S. Flier, and C. S. Mantzoros. 2002. Effects of Acute and Chronic Administration of the Melanocortin Agonist MTII in Mice with Diet-Induced Obesity. Diabetes 51: 1337-1345.

Pipeleers, D. G., F. C. Schuit, P. A. IN'T Veld, E. Maes, E. L. Hoogie-Peters, M. Van De Winkel, and W. Gepts. 1985. Interplay of nutrients and hormones in the regulation of insulin release. Endocrinology 117: 824-833.

Plum, L., M. Schubert, and J. C. Bruning. 2005. The role of insulin receptor signalling in the brain. Trends in Endocrinology and Metabolism: 16: 59-65.

Polkowska, J., and A. Gładysz. 2001. Effect of food manipulation on the neuropeptide Y neuronal system in the diencephalon of ewes. Journal of Chemical Neuroanatomy 21: 149-159.

Polonsky, K. S., B. D. Given, and E. Van Cauter. 1988. Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. Journal of Clinical Investigation 81: 442-448.

Popovic, V., D. Micic, S. Danjanovic, S. Zoric, M. Djurovic, S. Obradovic, M. Petakov, C. Dieguez, and F. F. Casanueva. 1998. Serum leptin and insulin concentrations in patients with insulinoma before and after surgery. European Journal of Endocrinology 138: 86-88.

Power, M. L., and J. Schulkin. 2008. Anticipatory physiological regulation in feeding biology: Cephalic phase responses. Appetite 50: 194-206.

Powley, T. L., and H. R. Berthoud. 1985. Diet and cephalic phase insulin responses. American Journal of Clinical Nutrition 42: 991-1002.

Pritchard, L. E., A. V. Turnbull, and A. White. 2002. Pro-opiomelanocortin processing in the hypothalamus: impact on melanocortin signalling and obesity. The Journal of Endocrinology 172: 411-421.

Prolo, P., M. L. Wong, and J. Licinio. 1998. Leptin. The International Journal of Biochemistry and Cell Biology 30: 1285-1290.

Pronchuk, N., A. G. Beck-Sickinger, and W. F. Colmers. 2002. Multiple NPY receptors Inhibit GABA synaptic responses of rat medial parvocellular effector neurons in the hypothalamic paraventricular nucleus. Endocrinology 143: 535-543.

Rabe, K., M. Lehrke, K. G. Parhofer, and U. C. Broedl. 2008. Adipokines and insulin resistance. Molecular Medicene 14: 741-751.

Rauw, W. M., E. Kanis, E. N. Noordhuizem Stassen, and F. J. Grommers. 1998. Undesirable side effects of selection for high production efficiency in farm animals: a review. Livestock Production Science 56: 15-33.

Rehfeld, J. F. 1998. Accurate measurement of cholecystokinin in plasma. Clinical Chemistry 44: 991-1001.

Rehfeld, J. F., G. Sun, T. Christensen, and J. G. Hillingso. 2001. The predominant cholecystokinin in human plasma and intestine is cholecystokinin-33. The Journal of Clinical Endocrinology and Metabolism 86: 251-258.

Reid, C. S. W. and J. B. Cornwall. 1959. The mechanical activity of the reticulo-rumen of cattle. Proceedings of New Zealand Society of Animal Production 23: 169-188.

Remppis, S., H. Steingass, L. Gruber, and H. Schenkel. 2011. Effects of energy intake on performance, mobilization and retention of body tissue, and metabolic parameters in dairy cows with special regard to effects of pre-partum nutrition on lactation: a review. Asian-Australian Journal of Animal Science 24: 540-572.

Reynolds, C. K. 1992. Metabolism of Nitrogenous Compounds by Ruminant Liver. The Journal of Nutrition 122: 850-854.

Reynolds, C. K., G. B. Huntington, H. F. Tyrrell, and P. J. Reynolds. 1988. Net metabolism of volatile fatty acids, D-β-hydroxybutyrate, nonesterified fatty acids, and blood gasses by portal-drained viscera and liver of lactating Holstein cows 1, 2. Journal of Dairy Science 71: 2395-2405.

Roche, J. F., D. Mackey, and M. D. Diskin. 2000. Reproductive management of postpartum cows. Animal Reproduction Science 60-61: 703-712.

Roche, J. R., P. G. Dillon, C. R. Stockdale, L. H. Baumgard, and M. J. VanBaale. 2004. Relationships among international body condition scoring systems. Journal of Dairy Science 87: 3076-3079.

Roche, J. R. 2006. Dry matter intake precalving in cows offered fresh and conserved pasture. Journal of Dairy Research 73: 273-276.

Roche, J. R., D. P. Berry, and E. S. Kolver. 2006a. Holstein-Friesian strain and feed effects on milk production, body weight and body condition score profiles in grazing dairy cows. Journal of Dairy Science 89: 3532-3543.

Roche, J. R., A. J. Sheahan, L. M. Chagas, and D. P. Berry. 2006b. Short communication: genetic selection for milk production increases plasma ghrelin in dairy cows. Journal of Dairy Science 89: 3471-3475.

Roche, J. R., D. P. Berry, J. M. Lee, K. A. Macdonald, and R. C. Boston. 2007a. Describing the body condition score change between successive calvings: A novel strategy generalizable to diverse cohorts. Journal of Dairy Science 90: 4378-4396.

Roche, J. R., A. J. Sheahan, L. M. Chagas, and D. P. Berry. 2007b. Concentrate supplementation reduces postprandial plasma ghrelin in grazing dairy cows: a possible neuroendocrine basis for reduced pasture intake in supplemented cows. Journal of Dairy Science 90: 1354-1363.

Roche, J. R., D. Blache, D. R. Miller, J. K. Kay, A. J. Sheahan, and D. W. Miller. 2008a. Neuroendocrine and physiological regulation of intake in domesticated ruminants: a review. Nutrition Research Review 21: 207-234.

Roche, J. R., A. J. Sheahan, L. M. Chagas, D. Blache, D. P. Berry, and J. K. Kay. 2008b. Long-term infusions of ghrelin and obestatin in early lactation dairy cows. Journal of Dairy Science 91: 4728-4740.

Roche, J. R., A. J. Sheahan, L. M. Chagas, and R. C. Boston. 2008c. Short communication: change in plasma ghrelin in dairy cows following an intravenous glucose challenge. Journal of Dairy Science 91: 1005-1010.

Roche, J. R., N. C. Friggens, J. K. Kay, M. W. Fisher, K. J. Stafford, and D. P. Berry. 2009. Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. Journal of Dairy Science 92: 5769-5801.

Rodgers, R. J., Y. Ishii, J. C. Halford, and J. E. Blundell. 2002. Orexins and appetite regulation. Neuropeptides 36: 303-325.

Rook, A. J., C. A. Huckle, and P. D. Penning. 1994. Effects of sward height and concentrate supplementation on ingestive behaviour of spring calving dairy cows grazing grass-clover swards. Applied Animal Behavioural Science 40: 101-112.

Ross, E., P. Moate, C. Bath, S. Davidson, T. Sawbridge, K. Guthridge, B. Cocks, and B. Hayes. 2012. High throughput whole rumen metagenome profiling using untargeted massively parallel sequencing. BMC Genetics 13: 53.

Rossi, M., M. S. Kim, D. G. Morgan, C. J. Small, C. M. Edwards, D. Sunter, S. Abusnana, A. P. Goldstone, S. H. Russell, S. A. Stanley, D. M. Smith, K. Yagaloff, M. A. Ghatei, and S. R. Bloom. 1998. A C-terminal fragment of Agouti-related protein increases feeding and antagonizes the effect of alpha-melanocyte stimulating hormone in vivo. Endocrinology 139: 4428-4431.

Ruderman, N. B. and M. N. Goodman. 1973. Regulation of ketone body metabolism in skeletal muscle. The American Journal of Physiology 224: 1391-1397.

Russell, J. B. and R. B. Hespell. 1981. Microbial rumen fermentation. Journal of Dairy Science 64: 1153-1169.

Russell, J. R. and T. Hino. 985. Regulation of lactate production in *Streptococcus bovis*: a spiralling effect that contributes to rumen acidosis. Journal of Dairy Science 68: 1712-1721.

Russell, J. B. and H. J. Strobel. 1987. Concentration of ammonia across cell membranes of mixed rumen bacteria. Journal of Dairy Science 70: 970-976.

Russell, J. B. and D. B. Wilson. 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? Journal of Dairy Science 79: 1503-1509.

Rutter, S. M., R. J. Orr, P. D. Penning, N. H. Yarrow, and R. A. Champion. 2002a. Ingestive behaviour of heifers grazing monocultures of ryegrass or white clover. Applied Animal Behaviour Science 76: 1-9.

Rutter, S. M., V. Tainton, R. A. Champion, and P. Le Grice. 2002b. The effect of a total solar eclipse on the grazing behaviour of dairy cattle. Applied Animal Behaviour Science 79: 273-283.

Sabine, J. R. and B. Connor Johnson. 1964. Acetate metabolism in the ruminant. Journal of Biological Chemistry 239: 89-93.

Sahu, A. and S. P. Kalra. 1993. Neuropeptidergic regulation of feeding behaviour Neuropeptide Y. T rends in Endocrinology and Metabolism 4: 217-224.

Sakurai, T. 2003. Orexin: a link between energy homeostasis and adaptive behaviour. Current Opinion in Clinical Nutrition and Metabolic Care 6: 353-360.

Sakurai, T., A. Amemiya, M. Ishii, I. Matsuzaki, R. M. Chemelli, H. Tanaka, S. C. Williams, J. A. Richarson, G. P. Kozlowski, S. Wilson, J. R. Arch, R. E. Buckingham, A. C. Haynes, S. A. Carr, R. S. Annan, D. E. McNulty, W. S. Liu, J. A. Terrett, N. A. Elshourbagy, D. J. Bergsma, and M. Yanagisawa. 1998. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behaviour. Cell 92: 573-85.

Sanacora, G., M. Kershaw, J. A. Finkelstein, and J. D. White. 1990. Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food deprivation. Endocrinology 127: 730-737.

Scaglia, G., H. T. Boland, and W. E. Wyatt. 2009. Effects of time of supplementation on beef stocker calves grazing ryegrass II. Grazing behaviour and dry matter intake. The Professional Animal Scientist 25: 749-756.

Schofield, P. 2000. Gas Production Methods. Farm Animal Metabolism and Nutrition. CABI Publishing, Oxon, UK.

Schütz, K. E., A. R. Rogers, Y. A. Poulouin, N. R. Cox, and C. B. Tucker. 2010. The amount of shade influences the behaviour and physiology of dairy cattle. Journal of Dairy Science 93: 125-133.

Schwartz, M. W., J. L. Marks, A. J. Sipols, D. G. Baskin, S. C. Woods, S. E. Kahn, and D. Porte, Jr. 1991. Central insulin administration reduces neuropeptide Y mRNA expression in the arcuate nucleus of food-deprived lean (Fa/Fa) but not obese (fa/fa) Zucker rats. Endocrinology 128: 2645-2647.

Schwartz, M. W., R. J. Seeley, L. A. Campfield, P. Burn, and D. G. Baskin. 1996. Identification of targets of leptin action in rat hypothalamus. Journal of Clinical Investigations 98: 1101-1106.

Schwartz, M. W., R. J. Seeley, S. C. Woods, D. S. Weigle, L. A. Campfield, P. Burn, and D. G. Baskin. 1997. Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. Diabetes 46: 2119-2123.

Schwartz, M. W., A. J. Sipols, J. L. Marks, G. Sanacora, J. D. White, A. Scheurink, S. E. Kahn, D. G. Baskin, S. C. Woods, D. P. Figlewicz, and et al. 1992. Inhibition of hypothalamic neuropeptide Y gene expression by insulin. Endocrinology 130: 3608-3616.

Schwartz, M. W., S. C. Woods, D. Porte, R. J. Seeley, and D. G. Baskin. 2000. Central nervous system control of food intake. Nature 404: 661-671.

Sedlackova, D., J. Kopeckova, H. Papezova, S. Vybiral, H. Kvasnickova, M. Hill, and J. Nedvidkova. 2011. Changes of plasma obestatin, ghrelin and NPY in anorexia and bulimia nervosa patients before and after a high-carbohydrate breakfast. Physiological Research Academia Scientiarum Bohemoslovaca 60: 165-173.

Seeley, R. J., C. J. Payne, and S. C. Woods. 1995. Neuropeptide Y fails to increase intraoral intake in rats. American Journal of Physiology, Regulatory, Integrative and Comparative Physiology 268: 423-427.

Seoane, J. R., C. A. Baile, and R. H. Martin. 1972. Humoral factors modifying feeding behaviour of sheep. Physiology and Behaviour 8: 993-995.

Shazly, K. 1952. Degradation of protein in the rumen of sheep 1. Some volatile fatty acids, including branched -chain isomers, found in vivo. Journal of Biochemistry 51: 640-647.

She, P., A. R. Hippen, J. W. Young, G. L. Lindberg, D. C. Beitz, L. F. Richardson, and R. W. Tucker. 1999. Metabolic responses of lactating dairy cows to 14-day intravenous infusions of glucagon. Journal of Dairy Science 82: 1118-1127.

Sheahan, A. J., E. S. Kolver and J. R. Roche. 2011. Genetic strain and diet effects on grazing behaviour, pasture intake, and milk production. Journal of Dairy Science 94: 3583-3591.

Sheahan, A. J., R. C. Boston, and J. R. Roche. 2013a. Diurnal patterns of grazing behaviour and humoral factors in supplemented dairy cows. Journal of Dairy Science 96: 3201-3210.

Sheahan, A. J., S. J. Gibbs and J. R. Roche. 2013b. Timing of supplementation alters grazing behaviour and milk production response in dairy cows. Journal of dairy Science 96: 477-483.

Sheikh, S. P., J. J. Holst, T. Skak-Nielsen, U. Knigge, J. Warberg, E. Theodorsson-Norheim, T. Hokfelt, J. M. Lundberg, and T. W. Schwartz. 1988. Release of NPY in pig pancreas: dual parasympathetic and sympathetic regulation. American Journal of Physiology - Gastrointestinal and Liver Physiology 255: 46-54.

Shiiya, T., M. Nakazato, M. Mizuta, Y. Date, M. S. Mondal, M. Tanaka, S.-I. Nozoe, H. Hosoda, K. Kangawa, and S. Matsukura. 2002. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. Journal of Clinical Endocrinology and Metabolism 87: 240-244.

Sipols, A. J., D. G. Baskin, and M. W. Schwartz. 1995. Effect of intracerebroventricular insulin infusion on diabetic hyperphagia and hypothalamic neuropeptide gene expression. Diabetes 44: 147-151.

Small, C. J., K. Wynne, and S. R. Bloom. 2009. The gut as a second brain. Peptides in Energy Balance and Obestiy. CABI, Oxfordshire, UK, pp 93-113.

Smith, M. S. 1993. Lactation alters neuropeptide-Y and proopiomelanocortin gene expression in the arcuate nucleus of the rat. Endocrinology 133: 1258-1265.

Sorensen, A., C. L. Adam, P. A. Findlay, M. Marie, L. Thomas, M. T. Travers, and R. G. Vernon. 2002. Leptin secretion and hypothalamic neuropeptide and receptor gene expression in sheep. American Journal of Physiology, Regulatory, Integrative and Comparative Physiology 282: 1227-1235.

Soriano, F. D., C. E. Polan, and C. N. Miller. 2000. Milk production and composition, rumen fermentation parameters, and grazing behaviour of dairy cows supplemented with different forms and amounts of corn grain. Journal of Dairy Science 83: 1520-1529.

Sporndly, E. 1991. Supplementation of dairy cows offered freshly cut herbage ad libitum with starchy concentrates based on barley or fibrous concentrates based on unmolassed sugar beet pulp and wheat bran. Journal of Agricultural Research 21: 131-139.

Stakelum, G. 1996. Practical grazing management for dairy cows. Irish Grassland and Animal Production Association Journal 30: 33-45.

Stakelum, G., and P. Dillon. 1988. The effect of concentrate type on herbage intake of high yielding grazing dairy cows. In: Proceedings of the 12th general meeting of the European Grassland Federation, Dublin, Ireland. pp 143-147.

Stangassinger, M., and D. Giesecke 986. Splanchnic metabolism of glucose and related energy substrates. Prentice Hall, Englewood Cliffs, NJ.

Stanley, B. G., S. E. Kyrkouli, S. Lampert, and S. F. Leibowitz. 1986. Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. Peptides 7: 1189-1192.

Stanley, S., K. Wynne, B. McGowan, and S. Bloom. 2005. Hormonal regulation of food intake. Physiological Reviews 85: 1131-1158.

Steinhour, W. D., and D. E. Bauman, 1988. Propionate metabolism: A new interpretation. Aspects of Digestive Physiology in Ruminants. Comstock Publishers, Ithaca, USA.

Steppan, C. M., S. T. Bailey, S. Bhat, E. J. Brown, R. R. Banerjee, C. M. Wright, H. R. Patel, R. S. Ahima, and M. A. Lazar. 2001. The hormone resistin links obesity to diabetes. Nature 409: 307-312.

Stevens, C. E., A. Sellers and F. A. Spurrell. 1960. Function of the bovine omasum in ingesta transfer. American Journal of Physiology 198: 449.

Stewart, C. S. 1977. Factors affecting the cellulolytic activity of rumen contents. Applied Environmental Microbiology 33: 497-502.

Stewart, W. E., D. G. Stewart and L. H. Schultz. 1958. Rates of volatile fatty acid production in the bovine rumen. Journal of Animal Science 17: 723-736.

Stobbs, T. H. 1973. The effect of plant structure on the intake of tropical pastures. Australian Journal of Agriculture Research 24: 809 - 819

Stockdale, C. R. 1985. Influence of some sward characteristics on the consumption of irrigated pastures. Grass and Forage Science 40: 31-39

Stockdale, C. R. 1999. The nutritive characteristics of herbage consumed by grazing dairy cows affect milk yield response obtained from concentrate supplementation. Australian Journal of Agriculture Research 39: 379-387.

Stockdale, C. R. 2000a. Effects of cereal grain, lupin-cereal grain or hay supplements on the intake and performance of grazing dairy cows. Australian Journal of Agriculture Research 39: 811-817.

Stockdale, C. R. 2000b. Levels of pasture substitution when concentrates are fed to grazing dairy cows in northern Victoria. Australian Journal of Experimental Agriculture 40: 913-921.

Sugino, T., Y. Hasegawa, Y. Kikkawa, J. Yamaura, M. Yamagishi, Y. Kurose, M. Kojima, K. Kangawa, and Y. Terashima. 2002a. A transient ghrelin surge occurs just before feeding in a scheduled meal-fed sheep. Biochemical and Biophysical Research Communications 295: 255-260.

Sugino, T., J. Yamaura, M. Yamagishi, A. Ogura, R. Hayashi, Y. Kurose, M. Kojima, K. Kangawa, Y. Hasegawa, and Y. Terashima. 2002b. A transient surge of ghrelin secretion before feeding is modified by different feeding regimens in sheep. Biochemical and Biophysical Research Communications 298: 785-788.

Sutton, J. D., M. S. Dhanoa, S. V. Morant, J. France, D. J. Napper, and E. Schuller. 2003. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. Journal of Dairy Science 86: 3620-3633.

Takahashi, H., Y. Kurose, S. Kobayashi, T. Sugino, M. Kojima, K. Kangawa, Y. Hasegawa, and Y. Terashima. 2006. Ghrelin enhances glucose-induced insulin secretion in scheduled meal-fed sheep. Journal of Endocrinology 189: 67-75.

Tartaglia, L. A. 1997. The Leptin Receptor. Journal of Biological Chemistry 272: 6093-6096.

Taweel, H. 2006. Improving dry-matter intake of perennial-ryegrass pasture by dairy cows. In: Fresh Herbage for Dairy Cattle. Springer. The Netherlands, pp 159-174.

Taweel, H. Z., B. M. Tas, J. Dijkstra, and S. Tamminga. 2004. Intake regulation and grazing behaviour of dairy cows under continuous stocking. Journal of Dairy Science 87: 3417-3427.

Taylor, W. H. 1959. Studies on gastric proteolysis 1. The proteolytic activity of human gastric juice and pig and calf gastric mucosal extracts below pH 5. Journal of Biochemistry 71: 73-83.

Teff, K. 2000. Nutritional implications of the cephalic-phase reflexes: endocrine responses. Appetite 34: 206-213.

Teff, K. L. and R. R. Townsend. 1999. Early phase insulin infusion and muscarinic blockade in obese and lean subjects. The American Journal of Physiology 277: 198-208.

ThanThan, S., T. Saito, S. Yannaing, H. Zhao, K. Nakashima, and H. Kuwayama. 2012. Glucagon-like peptide-1 inhibits insulinotropic effects of oxyntomodulin and glucagon in cattle. Domestic Animal Endocrinology 42: 155-164.

Thorne, P. L., J. G. Jago, E. S. Kolver, and J. R. Roche. 2003. Diet and genotype affect feeding behaviour of Holstein-Friesian dairy cows during late lactation. In: New Zealand Society of Animal Production. pp 124-127.

Thornton, J. E., C. C. Cheung, D. K. Clifton, and R. A. Steiner. 1997. Regulation of hypothalamic proopiomelanocortin mRNA by leptin in ob/ob mice. Endocrinology 138: 5063-5066.

Toshinai, K., M. S. Mondal, M. Nakazato, Y. Date, N. Murakami, M. Kojima, K. Kangawa, and S. Matsukura. 2001. Upregulation of ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. Biochemical and Biophysical Research Communications 281: 1220-1225.

Tozer, P. R., F. Bargo, and L. D. Muller. 2004. The effect of pasture allowance and supplementation on feed efficiency and profitability of dairy systems. Journal of Dairy Science 87: 2902-2911.

Trautman, A. and J. Fiebiger. 1952. Fundamentals of the histology of domestic animals. Comstock Publishers, Ithaca. USA.

Treacher, R. J., I. M. Reid, and C. J. Roberts. 1986. Effect of body condition at calving on the health and performance of dairy cows. Animal Production 43: 1-6.

Trevaskis, L. M., W. J. Fulkerson, and K. S. Nandra. 2004. Effect of time of feeding carbohydrate supplements and pasture on production of dairy cows. Livestock Production Science 85: 275-285.

Trinci, A. P. J., D. R. Davies, K. Gull, M. I. Lawrence, B. B. Nielsen, A. Rickers, and M. K. Theodorou. 1994. Anaerobic fungi in herbivorous animals. Mycological Research 98: 129-152.

Tschop, M., D. L. Smiley, and M. L. Heiman. 2000. Ghrelin induces adiposity in rodents Nature 407: 908-913.

Turek, F. W. and M. U. Gillette. 2004. Melatonin, sleep and circadian rhythms: rationale for development of specific melatonin agonists. Sleep Medicine 5: 523-532.

Turton, M. D., D. O'Shea, I. Gunn, S. A. Beak, C. M. Edwards, K. Meeran, S. J. Choi, G. M. Taylor, M. M. Heath, P. D. Lambert, J. P. Wilding, D. M. Smith, M. A. Ghatei, J. Herbert, and S. R. Bloom. 1996. A role for glucagon-like peptide-1 in the central regulation of feeding. Nature 379: 69-72.

Tylutki, T. P., D. G. Fox, V. M. Durbal, L. O. Tedeschi, J. B. Russell, M. E. Van Amburgh, T. R. Overton, L. E. Chase, and A. N. Pell. 2008. Cornell Net Carbohydrate and Protein System: A model for precision feeding of dairy cattle. Animal Feed Science and Technology 143: 174-202.

Ulyatt, M. J., G. C. Waghorn, A. John, C. S. W. Reid, and J. Monro. 1984. Effect of intake and feeding frequency on feeding behaviour and quantitative aspects of digestion in sheep fed chaffed lucerne hay. The Journal of Agricultural Science 102: 645-657.

Vahl, T. P., B. W. Paty, B. D. Fuller, R. L. Prigeon, and D. A. DaAlessio. 2003. Effects of GLP-1 (7-36) NH2, GLP-1 (7-37), and GLP-1- (9-36) NH2 on intravenous glucose tolerance and glucose-induced insulin secretion in healthy humans. Journal of Clinical Endocrinology and Metabolism 88: 1772-1779.

Van Amburgh, M. E., L. E. Chase, T. R. Overton, D. A. Ross, E. B. Recktenwald, R. J. Higgs, and T. P. Tylutki. 2010. Updates to the Cornell Net Carbohydrate and Protein System v6.1 and implications for ration formulation. In: Cornell Nutrition Conference, Syracuse NY. pp 144-159.

Van den Pol, A. N., J. P. Wuarin, and F. E. Dudek. 1990. Glutamate, the dominant excitatory transmitter in neuroendocrine regulation. Science 250: 1276-1278.

Van Soest, P. J. 1994. Intake. Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, NY. USA, pp 337-353.

Vasilatos, R. and P. J. Wangsness. 1980. Changes in concentrations of insulin, growth hormone and metabolites in plasma with spontaneous feeding in lactating dairy cows. The Journal of Nutrition 110: 1479-1487.

Veerkamp, R. F., G. Simm, and J. D. Oldham. 1994. Effects of interaction between genotype and feeding system on milk production, feed intake, efficiency and body tissue mobilization in dairy cows. Livestock Production Science 39: 229-241.

Verbyla, A. P., B. R. Cullis, M. G. Kenwood, and S. J. Welham. 1999. The analysis of designed experiments and longitudinal data by using smoothing splines. Applied Statistics 48: 269-311.

Vilsbøll, T., T. Krarup, S. Madsbad, and J. J. Holst. 2003. Both GLP-1 and GIP are insulinotropic at basal and postprandial glucose levels and contribute nearly equally to the incretin effect of a meal in healthy subjects. Regulatory Peptides 114: 115-121.

Wahlestedt, C. and D. J. Reis. 1993. Neuropeptide Y-related peptides and their receptors--are the receptors potential therapeutic drug targets? Annual Review of Pharmacology and Toxicology 33: 309-352.

Wales, W. J. and P. T. Doyle. 2003. Effect of grain and straw supplementation on marginal milk-production responses and rumen fermentation of cows grazing highly digestible subterranean clover pasture. Australian Journal of Experimental Agriculture 43: 467-474.

Wales, W. J., P. T. Doyle, and Y. J. Williams. 2001. Effect of grain supplementation and the provision of chemical or physical fibre on marginal milk production responses of cows grazing perennial ryegrass pastures. Australian Journal of Experimental Agriculture 41: 465-471.

Wales WJ, Stockdale CR, Doyle PT (2005) Plant and sward characteristics to achieve high intake in ruminants. In 'Utilisation of grazed grass in temperate animal systems: proceedings of a satellite workshop of the XXth International Grassland Congress, Cork, Ireland, July 2005 pp. 37–47.

Watson, S. I., X. Lu, D. Bagnol, G. Barsh, I. Gantz, and H. Akil. 1999. POMC and AgRP relationships and complexities. In: Proceedings of the American Neuroendocrine Society Neuroendocrine Workshop, San Diego, CA. pp 32.

Webb, S. D. 1998. Hornless ruminants. In: Evolution of tertiary mammals of North America: Terrestrial carnivores, ungulates, and ungulate like mammals. C. M. Janis, K. M. Scott and L. L. Jacobs. No. 1. Cambridge University Press, Cambridge, UK. pp 463-476

Weidemann, M. J. and H. A. Krebs. 1969. The fuel of respiration of rat kidney cortex. Biochemical Journal 112: 149-166.

Weigand, E., J. W. Young, and A. D. McGilliard. 1975. Volatile fatty acid metabolism by rumen mucosa from cattle fed hay or grain. Journal of Dairy Science 58: 1294-1300.

Weimer, P. J. 1992. Cellulose degradation by ruminal microorganisms. Critical Reviews in Biotechnology 12: 189-223.

Weimer, P. J. 1996. Why don’t ruminal bacteria digest cellulose faster? Journal of Dairy Science 79: 1496-1502.

Weimer, P. J., G. C. Waghorn, C. L. Odt, and D. R. Mertens. 1 999. Effect of diet on populations of three species of ruminal cellulolytic bacteria in lactating dairy cows. Journal of Dairy Science 82: 122-134.

Weir, G. C., S. Mojsov, G. K. Hendrick, and J. F. Habener. 1989. Glucagon like peptide I (7-37) actions on endocrine pancreas. Diabetes 38: 338-342.

Wells, J. E. and J. B. Russell. 1996. Why do many ruminal bacteria die and lyse so quickly? Journal of Dairy Science 79: 1487-1495.

Wertz-Lutz, A. E., T. J. Knight, R. H. Pritchard, J. A. Daniel, J. A. Clapper, A. J. Smart, A. Trenkle, and D. C. Beitz. 2006. Circulating ghrelin concentrations fluctuate relative to nutritional status and influence feeding behaviour in cattle. Journal of Animal Science 84: 3285-3300.

West, C. E., and R. F. Passey. 1967. Effect of glucose load and of insulin on the metabolism of glucose and of palmitate in sheep. Journal of Biochemistry 102: 58-64.

Willesen, M. G., P. Kristensen, and J. Romer. 1999. Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. Neuroendocrinology 70: 306-316.

Williams, G., C. Bing, X. J. Cai, J. A. Harrold, P. J. King, and X. H. Liu. 2001. The hypothalamus and the control of energy homeostasis: Different circuits, different purposes. Physiology and Behaviour 74: 683-701.

Williams, G., J. S. Gill, Y. C. Lee, H. M. Cardoso, B. E. Okpere, and S. R. Bloom. 1989. Increased neuropeptide Y concentrations in specific hypothalamic regions of streptozocin-induced diabetic rats. Diabetes 38: 321-327.

Williams, Y. J., W. J. Wales, P. T. Doyle, A. R. Egan, and C. R. Stockdale. 2005a. Effects of grain or hay supplementation on the chewing behaviour and stability of rumen fermentation of dairy cows grazing perennial rye-grass-based pasture in spring. Australian Journal of Experimental Agriculture 45: 1519-1528.

Williams, Y. J., G. P. Walker, P. T. Doyle, A. R. Egan, and C. R. Stockdale. 2005b. Rumen fermentation characteristics of dairy cows grazing different allowances of Persian clover- or perennial ryegrass-dominant swards in spring. Australian Journal of Experimental Agriculture 45: 665-675.

Williamson, J. R. and H. A. Krebs. 1961. Acetoacetate as fuel of respiration in the perfused rat heart. Journal of Biochemistry 80: 540-547.

Wilson, B. D., M. M. Ollmann, and G. S. Barsh. 1 999. The role of agouti-related protein in regulating body weight. Molecular Medicine Today 5: 250-256.

Wolfgang, M. J. and M. D. Lane. 2006. The role of hypothalamic malonyl-CoA in energy homeostasis. Journal of Biological Chemistry 281: 37265-37269.

Woods, S. and D. Porte. 1977. Relationship between plasma and cerebrospinal fluid insulin levels of dogs. American Journal of Physiology - Gastrointestinal and Liver Physiology 233: 331-334.

Woods, S. C., E. C. Lotter, L. D. McKay, and D. Porte, Jr. 1979. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. Nature 282: 503-505.

Woods, S. C., T. A. Lutz, N. Geary, and W. Langhans. 2006. Pancreatic signals controlling food intake; insulin, glucagon and amylin. Philosophical Transactions of the Royal Society B: Biological Sciences 361: 1219-1235.

Wren, A. M., L. J. Seal, M. A. Cohen, A. E. Brynes, G. S. Frost, K. G. Murphy, W. S. Dhillo, M. A. Ghatei, and S. R. Bloom. 2001a. Ghrelin enhances appetite and increases food intake in humans. The Journal of Clinical Endocrinology and Metabolism 86: 5992.

Wren, A. M., C. J. Small, C. R. Abbott, W. S. Dhillo, L. J. Seal, M. A. Cohen, R. L. Batterham, S. Taheri, S. A. Stanley, M. A. Ghatei, and S. R. Bloom. 2001b. Ghrelin causes hyperphagia and obesity in rats. Diabetes 50: 2540-2547.

Wren, A. M., C. J. Small, H. L. Ward, K. G. Murphy, C. L. Dakin, S. Taheri, A. R. Kennedy, G. H. Roberts, D. G. Morgan, M. A. Ghatei, and S. R. Bloom. 2000. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 141: 4325-4328.

Wyburn, R. S. 1980. The mixing and propulsion of the stomach contents of ruminants. Digestive Physiology and Metabolism in Ruminants. MTP Press, Lancaster, England.

Wynne, K., S. Stanley, and S. Bloom. 2004. The gut and regulation of body weight. The Journal of Clinical Endocrinology and Metabolism 89: 2576-2582.

Yamanaka, A., T. Sakurai, T. Katsumoto, M. Yanagisawa, and K. Goto. 1999. Chronic intracerebroventricular administration of orexin-A to rats increases food intake in daytime, but has no effect on body weight. Brain Research 849: 248-252.

Yamauchi, T., J. Kamon, H. Waki, Y. Imai, N. Shimozawa, K. Hioki, S. Uchida, Y. Ito, K. Takakuwa, J. Matsui, M. Takata, K. Eto, Y. Terauchi, K. Komeda, M. Tsunoda, K. Murakami, Y. Ohnishi, T. Naitoh, K. Yamamura, Y. Ueyama, P. Froguel, S. Kimura, R. Nagai, and T. Kadowaki. 2003. Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. Journal of Biological Chemistry 278: 2461-2468.

Yang, W. S., W. J. Lee, T. Funahashi, S. Tanaka, Y. Matsuzawa, C. L. Chao, C. L. Chen, T. Y. Tai, and L. M. Chuang. 2001. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. The Journal of Clinical Endocrinology and Metabolism 86: 3815-3819.

Yang, Y. K., D. A. Thompson, C. J. Dickinson, J. Wilken, G. S. Barsh, S. B. Kent, and I. Gantz. 1999. Characterization of Agouti-related protein binding to melanocortin receptors. Molecular Endocrinology 13: 148-155.

Zeder, M. A. and B. Hesse. 2000. The initial domestication of goats (Capra hircus) in the Zagros mountains 10,000 years ago. Science 287: 2254-2257.

Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold, and J. M. Friedman. 1994. Positional cloning of the mouse obese gene and its human homologue. Nature 372: 425-432.